

Biology



Cells & exchanges

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1

The cell as the basic unit of structure in prokaryotic and eukaryotic organisms

Contents

Prokaryotic cells

- ▼ The main features of prokaryotic cells

Eukaryotic cells

- ▼ The structure of plant and animal cells as seen through an optical microscope
- ▼ The preparation of temporary mounts, the use of simple staining techniques (see practical work) and the estimation of size (also see practical work)

Introduction

All living organisms are composed of cells. It is calculated that an average human adult is made up of around 10^{14} cells, each coordinated with the others to form functional tissues, organs and systems. At the other extreme, some organisms are composed of a single cell; Amoeba, bacteria and yeast are examples of single celled organisms.

The word 'cell' to describe living units was first coined by the English Scientist Robert Hooke who, in the 1660s, used a simple microscope to observe the contents of a slice of cork from the bark of a tree. He noted that the structure resembled a multitude of tiny compartments like the cells inhabited by monks in a monastery - hence the term 'cell'.

Cells are grouped into two major types, **prokaryotic** and **eukaryotic**, the most important distinction between them being the presence or absence of a nucleus. The word '*karyon*' in Greek means 'nucleus', '*eu*' means true, and '*pro*' means 'before'. Eukaryotic cells are so named because they have a true nucleus and prokaryotic cells are built on an earlier design without a true nucleus.

PROKARYOTIC CELLS

The earliest life forms on the planet were prokaryotes similar in structure to present day bacteria. Prokaryotes are all single celled organisms and they are classified in a separate kingdom called **Kingdom Prokaryotae** (formerly, Monera). Although they are all constructed according to the same basic pattern, they are a very diverse group with individual types capable of inhabiting parts of the earth as distinct from each other as hot sulphurous springs and permafrost. Some prokaryotes are photosynthetic and may occur in such numbers as to form a blue green scum on the surface of stagnant water. Others (decomposers) live off the dead remains of other organisms, assisting in the breakdown, decay and recycling of essential nutrients. A few prokaryotes have gained notoriety as disease agents (pathogens) due to their parasitic life style e.g. *Mycobacterium tuberculosis*, *Salmonella enteritidis*.

Prokaryotic cells are much smaller than eukaryotic cells, ranging from 1-10 µm in size (eukaryotic cells range from 10-100µm). Like eukaryotic cells, prokaryotes are capable of carrying out the life processes of sensitivity (reacting to different stimuli e.g. temperature and interacting with other cells), movement (all living cells show streaming of the cytoplasm even if the cell itself does not move), nutrition, respiration (release and use energy from energy rich substances), excretion (elimination of toxic waste products), growth (increase in size and complexity), and reproduction.

Not all prokaryotes are able to move, but some possess **flagella**, which are like elongated cilia (but without the microtubules - see 10.2), capable of propelling the organism through water. The cell wall is not made of cellulose, but is formed from a mixture of polysaccharides and amino acids called **murein**. Many bacteria which cause disease (pathogenic bacteria) have a **slime capsule** as their outermost layer which helps in protection against the body defences of the infected organism.

Prokaryotic cells generally divide and reproduce themselves much more quickly than eukaryotic cells. When grown in ideal conditions, a typical bacterial cell can grow and divide every 20 minutes, so a single cell could theoretically give rise to 2^{36} (that is a 2 with 36 noughts) in just 12 hours!

The structure of a generalised bacterial cell is illustrated and described in section 10.2 which also lists the main differences between prokaryotic and eukaryotic cells in the form of a table.

◆ CHECKPOINT SUMMARY

- ◆ Prokaryotic cells (bacteria) lack a true nucleus and membrane bound organelles
- ◆ The DNA is located in a central strand, and as circular plasmids in the rest of the cytoplasm
- ◆ The cell wall is not cellulose as in plant cells.
- ◆ Respiration is located in mesosomes which are infoldings of the cell surface membrane, showing the principle of increased surface area of membranes with true mitochondria
- ◆ The origin of mitochondria and chloroplasts as mutualistic prokaryotes with eukaryote cells, explains their absence in prokaryotes
- ◆ Ribosomes present in both
- ◆ Prokaryotic cells may form colonies but never multicellular organisms/tissues.

EUKARYOTIC CELLS

The structure of plant and animal cells as seen through an optical microscope.

Hooke used the word cell to mean a small compartment. When a slice of tissue from a plant or animal is viewed through a microscope, it is easy to see how this idea originated. Plant cells are generally easier to see under the microscope, as the cell membrane of each cell is surrounded by a relatively thick **cellulose cell wall** which gives each cell a more visible boundary.

The plant cell wall is made of a fibrous cellulose framework mixed with other materials, notably **pectin**. Pectin is a glue like material (it is used to make jam set) and it is concentrated on the surface, sticking adjacent

cells together. This sticky region can be seen as a line on electron micrographs and is called the middle lamella. When stretched around an expanded (turgid) cell, the cell wall makes a considerable contribution to the support of non-woody structures, e.g. leaves. The cell wall is fully permeable to water and dissolved solutes, but influences the exchange of these across the partially permeable cell membrane, by exerting various physical and chemical forces. The wall is perforated by tiny holes, through which strands of cytoplasm (**plasmodesmata**) pass giving direct communication between neighbouring cells.

In 'woody' parts the cell wall is impregnated with **lignin**. Lignin is a hard, impermeable substance, and lignification typically results in the death of the cell. Such dead lignified tissues are specialised for water transport (e.g. xylem) and support (e.g. xylem and sclerenchyma fibres).

As cells in a multicellular organism become more specialised (differentiated) for particular activities e.g. transmission of impulses in nerve cells, they lose the ability to carry out some of the life processes, e.g. under normal circumstances most nerve cells lose the ability to divide. However, if some of these differentiated cells are removed from the body and kept isolated in a special culture solution they are capable of surviving on their own, and may even regain some of the lost abilities, such as the ability to divide.

Multicellular organisms may also produce single cells which can live independently, e.g. spermatozoa.

Cells which lose their nucleus cannot survive or divide, because the nucleus controls all the life processes. If an Amoeba is cut into two, the part without the nucleus dies, but the part with the nucleus lives, grows and reproduces.

Cheek lining (squamous) cell under light microscope

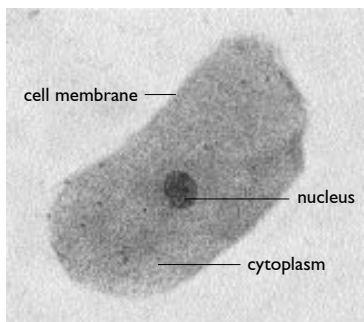
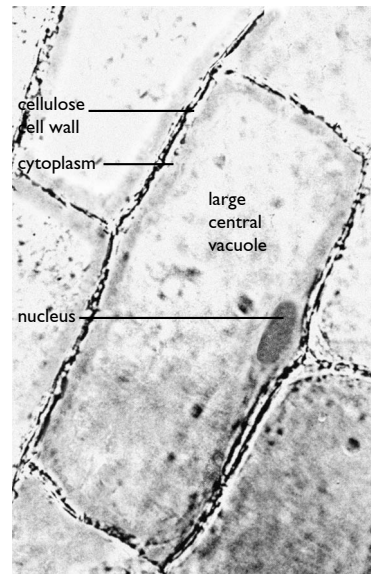


Table of differences between typical animal and plant cells seen under the light microscope.

Animal cells	Plant cells
Cell surface membrane	Cell surface membrane secretes cellulose cell wall
Small scattered vacuoles	Large central vacuole surrounded by membrane (tonoplast)
No chloroplasts	Chloroplasts with starch grains (in cells exposed to light)

Onion epidermis cells under light microscope



◆ CHECKPOINT SUMMARY

- ◆ Eukaryotes do have a true membrane bound nucleus containing chromosomes of DNA and histone proteins, and membrane bound organelles as described previously
- ◆ Eukaryotic cells typically form tissues in multicellular organisms
- ◆ Cell differentiation and specialisation prevent the easy definition of typical animal and plant cells
- ◆ 'Typical' cells would be those considered to show features common to the vast majority of cells in animals or plants
- ◆ A squamous (pavement) epithelium cell from the lining of the mouth could be considered as a 'typical' animal cell
- ◆ A mesophyll cell from a leaf could be considered as a 'typical' plant cell
- ◆ Under the best light microscope both the typical animal cell and the typical plant cell can be seen to possess a cell surface membrane, cytoplasm, nucleus with nucleolus, mitochondria, endoplasmic reticulum, and Golgi body
- ◆ In addition the typical plant cell can be seen to possess a cell wall around the cell surface membrane, a large central vacuole surrounded by the tonoplast membrane, and chloroplasts.

Estimating the size when observing cells through a microscope

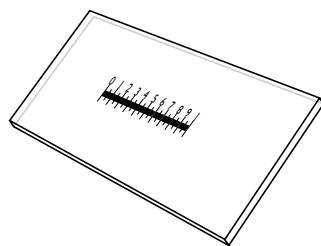
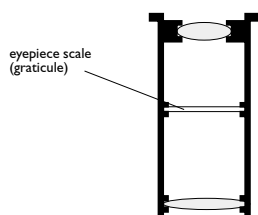
When looking at objects under the light microscope the magnification of the object as you see it can be calculated by multiplying the magnifying power of the eyepiece lens and the objective lens (the lens at the bottom) to get the overall magnification, for example $\times 10$ and $\times 40$ respectively under high power giving a total magnification of $\times 400$. However, when you make a drawing of a specimen seen under the light microscope, the size of your drawing further magnifies the image. To calculate the linear magnification (e.g. the number of times the length of an object has been magnified) of drawings it is necessary to measure your drawing and calculate how many times it is greater than the actual size of the object as seen under the microscope.

To calculate the actual size of the object you need to use a stage micrometer and an eye piece graticule. The stage micrometer is a microscope slide with a very fine scale marked on it. If it was possible to place a specimen on the scale it could be measured directly, but this is not easily done. Therefore a glass or plastic disc with a printed scale, known as an eye piece graticule is mounted in the microscope eyepiece. It does not have actual measurements on it, just lines. At each magnification the number of divisions on the eye piece graticule equal to a certain real measurement on the stage micrometer is noted. For example 10 eye piece divisions will equal a larger real measurement at $\times 100$ than at $\times 400$. Once this calibration has been carried out the eye piece graticule can be used to measure the specimen.

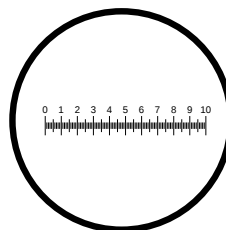
Cheek cells are about $50\text{ }\mu\text{m}$ in diameter, red blood cells $6\text{--}8\text{ }\mu\text{m}$, and some plant cells up to $500\text{ }\mu\text{m}$.

Measuring with a microscope eyepiece graticule

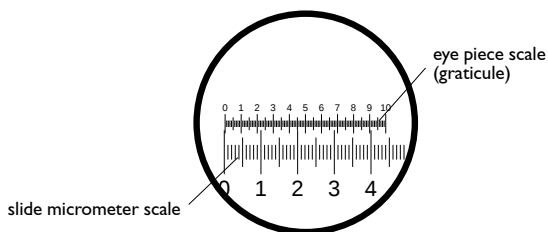
Eyepiece scale (graticule)



Scale in mm etched upon a microscope slide or a printed acetate sheet (slide micrometer)



Eyepiece scale as seen when held up to the light away from the microscope.



Accurate measurements can be achieved using the two scales in combination through the lenses of the microscope.

◆ CHECKPOINT SUMMARY

- ◆ If the specimen can be observed with the naked eye without the need for magnification the calculation of the linear magnification of any drawing of it is straightforward - being the number of times any linear dimension of the drawing is greater than that of the specimen
- ◆ Magnifications of images of slides of biological material are often given in terms relating to the magnification of the microscope image, e.g. $\times 100$ and $\times 400$
- ◆ However reproductions of the image as seen down a microscope introduce another layer of magnification which is often overlooked
- ◆ To calculate the linear magnification of drawings of microscope images of specimens it is necessary to know how many times the drawing is larger than the actual specimen
- ◆ The actual size of the specimen is measured with an eye piece graticule and stage micrometer.

2

The electron microscope and the technique of cell fractionation in the study of ultrastructure

Contents

Electron microscopes

- ▼ The difference between magnification and resolution.
- ▼ The principles and limitations of transmission and scanning electron microscopes

Cell ultrastructure

- ▼ Interpretation of electron micrographs (see teaching guides)
- ▼ Identification of the principal features and organelles of a eukaryotic cell. Cell wall and plasma membranes, nucleus, chloroplasts, mitochondria, lysosomes, ribosomes, endoplasmic reticulum, Golgi apparatus, microvilli and vesicles.
- ▼ The functions of these structures
- ▼ The principal features of a bacterium. Cell wall, capsule and genetic material

Cell fractionation

- ▼ Principles of cell fractionation and ultracentrifugation as used to separate cell components

ELECTRON MICROSCOPES

The difference between magnification and resolution

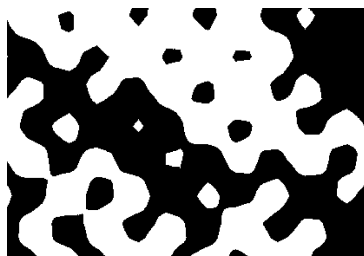
Magnification

For the visual study of cell structure it is necessary to enlarge (magnify) them by the use of microscopes. **Light microscopes** that you are most likely to come across in general Biology laboratories usually magnify up to 400 times (special techniques could take this up to 1000 times). However, to continue magnifying objects past a certain point reveals no further detail. In order to see more detail it is necessary to upgrade the resolving power (resolution).

Resolution

Resolution or resolving power can be defined as the ability of an optical system (e.g. the light microscope, the eye) to distinguish (resolve) detail in an image of an object.

The amount of detail that can be seen in an image is determined by the resolving power of the microscope system being used. In the case of looking at material under the light microscope the resolving power depends upon the light being able to distinguish this detail. To do



this light must be reflected back from structures. Any structures less than 0.2 micrometres (μm), that is 200 nanometres (nm) in diameter cannot be distinguished by light, nor can two structures closer than 0.2 μm be seen as separate.

(1 μm = one thousandth of a millimetre, 1 nm = one millionth of a millimetre)

To improve the resolution, it is necessary to have a device which uses a beam of electrons, instead of light, called the **electron microscope**. Electron beams have a much shorter wavelength than light and give a 100 times better resolution. The magnification of an electron microscope picture (electron micrograph) can also be increased significantly to reveal this extra detail. The most powerful electron microscope can magnify up to $\times 500\,000$.

The principles and limitations of transmission and scanning electron microscopes

There are two types of electron microscope. In the **transmission electron microscope (TEM)**, an extremely thin section of the specimen is held on a metal grid and an electron beam passes right through it. Inside the electron microscope tube, electrons are accelerated by high voltage and focussed into a fine beam by a strong electromagnet (condenser lens). The beam passes through the specimen and is focussed further by additional electromagnets, referred to as the objective, intermediate and projector lenses, to give an image on a fluorescent screen, similar to the screen of a television set.

Electrons are disrupted by particles in the specimen to produce lighter and darker areas on the screen which correspond to dense and less dense parts of the specimen. If a photographic film plate is substituted for the fluorescent screen, the resulting picture is called an **electron micrograph**. A vacuum is maintained inside the tube of the electron microscope so that the electron beam is not disrupted by molecules of the air e.g. oxygen and water. For this reason, it is impossible to view living specimens. This, along with the risk of the distortion of the delicate sub-microscopic structures (production of artefacts) by the preparation procedures are the principal limitations of electron microscope images. They show the structure of dead cells which have been subjected to mechanical and chemical trauma in the preparation process and are held in a vacuum.

Specimens are prepared by immersion in a series of increasingly pure solutions of alcohol which serve to dehydrate the cell components, then they are typically embedded in a block of resin which is cut into very thin sections by a machine resembling a high tech. bacon slicer called a microtome with a diamond or glass cutting surface. To improve the contrast of these pictures, it is common to 'stain' the specimen with the salts of heavy metals such as lead and uranium which increase the scattering of the electrons where the 'stains' are taken up.

In the **scanning electron microscope (SEM)**, the beam of electrons passes back and forth across the surface of the prepared specimen producing a three dimensional effect. This technique is used to show surface features.

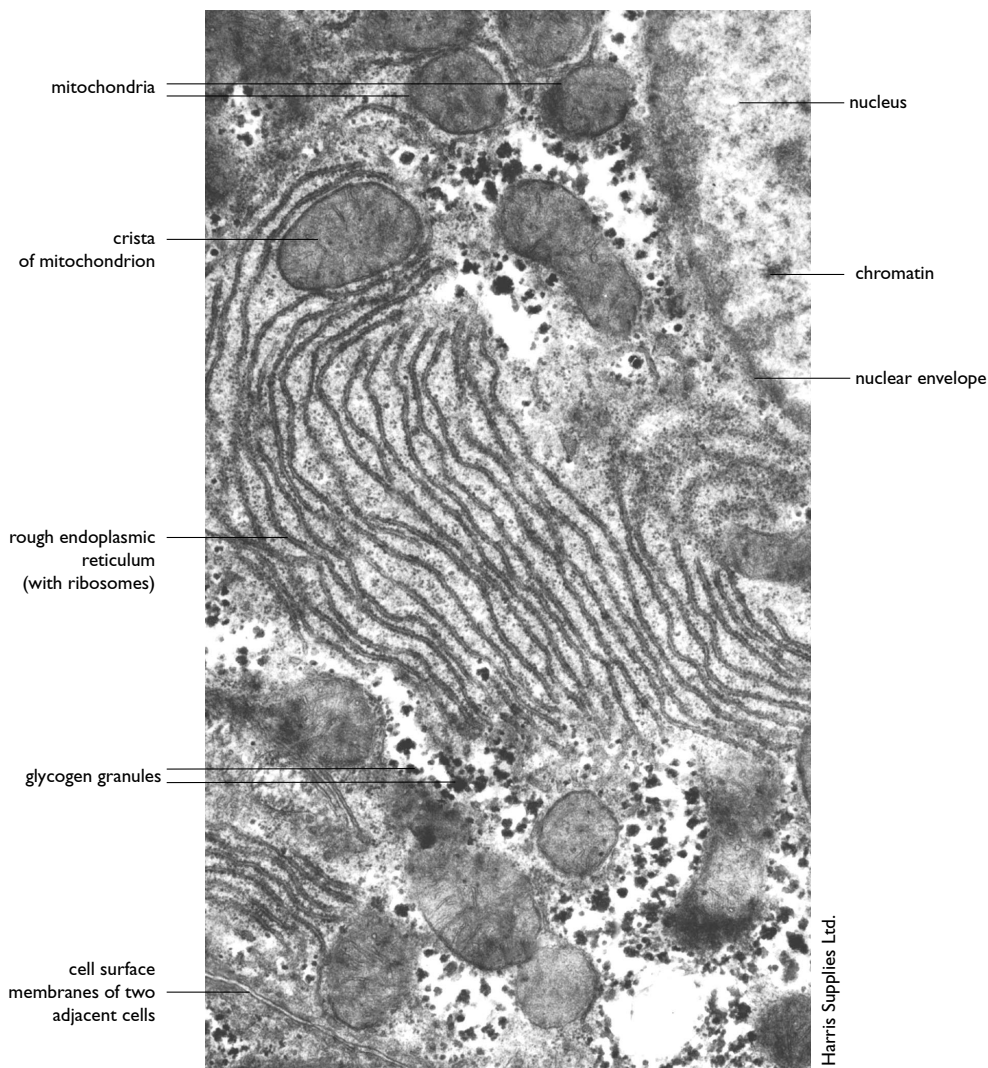
◆ CHECKPOINT SUMMARY

- ◆ Electron microscopes use a beam of electrons, instead of light. The electrons are focussed by magnets onto a fluorescent screen to produce an image which may be photographed to produce electronmicrographs
- ◆ Electron beams have a much shorter wavelength than light which reveals much more detail of the specimen, and allows useful magnification up to $\times 500\,000$
- ◆ Specimens must be completely dehydrated and mounted in a vacuum to avoid scattering of the electrons
- ◆ There are two types of electron microscope, the transmission electron microscope (TEM) and the scanning electron microscope (SEM)
- ◆ In the TEM, electrons pass through very thin sections which have been stained with salts of heavy metals such as lead and uranium to enhance differentiation between structures
- ◆ In the SEM, electrons are reflected off of the surfaces of specimens to give 3D images
- ◆ The major limitation of the use of electron microscopy is that the picture of the specimen is far removed from the reality of the living tissue or organism
- ◆ The drastic preparation of the specimen, including complete dehydration, very thin sectioning, staining, high temperatures and vacuum conditions, raises problems of the interpretation of the image, and the production of 'artificial' structures (artefacts)
- ◆ Resolution is the ability to see detail
- ◆ The higher the resolution the more of the detail present can be seen
- ◆ Magnification is enlarging in size
- ◆ Increasing magnification only reveals extra detail if the detail can be resolved
- ◆ Resolving power of light microscope limited by the wavelength of light.
- ◆ Put simply some detail is so small that it cannot be 'hit' by light, and therefore cannot be seen
- ◆ Endless magnification of the image produced by a light microscope yields no extra information
- ◆ Wavelengths of electron beams much shorter than that of light and give the electron microscope much greater resolving power than the light microscope
- ◆ Therefore extra magnification up to $\times 250\,000$ yields an equivalent amount of detail.

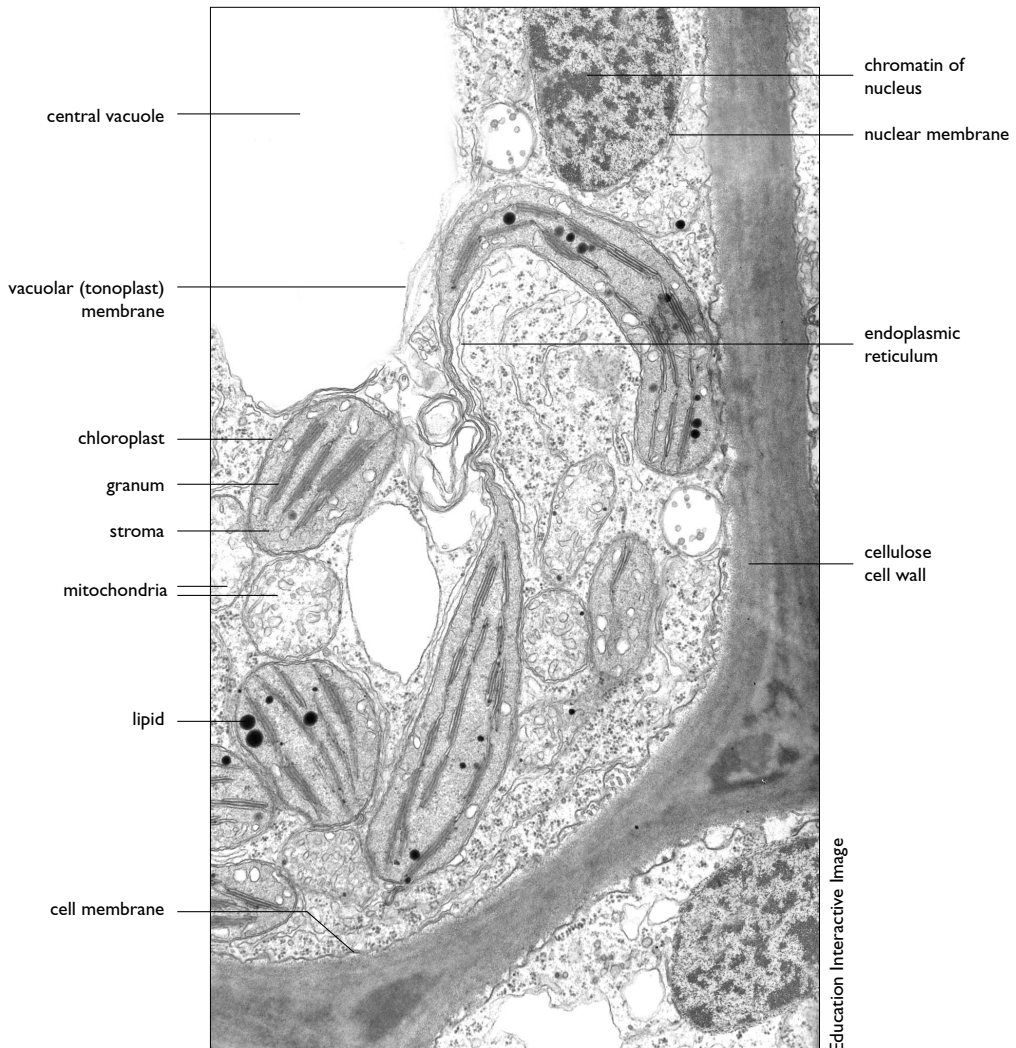
CELL ULTRASTRUCTURE

The electron microscope reveals a wealth of detail and shows the cell to be packed with a variety of structures, including **organelles**. Organelles are membrane lined compartments, found only in eukaryotic cells, and include the nucleus, mitochondria, chloroplasts and lysosomes. Each is characterised by the performance of a specific function or functions, for example, the mitochondria carry out the function of respiration. In prokaryotic cells these functions are not so localised.

EM part of an animal cell (liver)



EM part of plant leaf cell



Nucleus - the Control Centre

Even with the optical microscope, the nucleus is clearly visible, having an average diameter in animal cells (liver) of about $5\text{ }\mu\text{m}$, and in plant cells (mesophyll) of about $15\text{ }\mu\text{m}$. The nucleus stands out when cells are viewed under the microscope because of the material **chromatin**, a mixture of DNA and protein, which takes up the stains used in slide preparations. Chromatin is the material which forms the chromosomes just before nuclear division. The nucleus is surrounded by a double membrane, the **nuclear envelope** which has the same structure as, and is continuous with, the other membrane systems of the cell. Three or four thousand large pores (up to 100nm diameter) perforate the nuclear envelope, but plugs of protein and RNA appear to fill the pores.

Genes in the chromosomes direct all the cell's activities and determine its life span and reproductive cycle. The nucleus is rightly called the command centre, but it does not contain all of the genetic material of the cell. Mitochondria and chloroplasts also contain DNA and have genes of their own.

Nucleolus

This is an even more darkly staining region in the nucleus, which consists of a mass of RNA, used in the manufacture of **ribosomes**. Ribosomes are relatively small structures, about 20 nm in diameter which occur in large numbers, often several thousand per cell either bound to internal cell membranes or free in the cytoplasm. They are built in two sections called sub-units, made of approximately equal quantities of RNA and protein. They act as the sites of assembly for new proteins, manufactured both for export and for internal use.

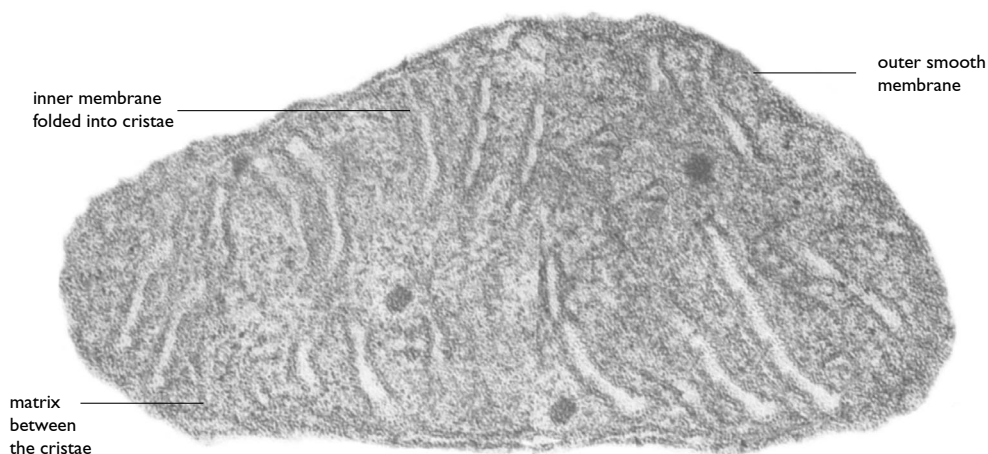
Mitochondria

Mitochondria (singular = mitochondrion) carry out aerobic respiration, which transfers energy from energy rich substances such as glucose, into the energy rich compound ATP. High energy consuming tissues like those in the muscles or brain may contain up to a thousand mitochondria in a single cell.

Mitochondria can vary greatly in length and shape, but are never greater than 1µm in diameter. There is an outer and an inner membrane. The area of the inner membrane is made as large as possible by infoldings of the membrane (**cristae**), and it is encrusted on its inner surface with spherical bodies which contain the enzymes for making ATP. The more active the cell, the more cristae and the more enzymes there are. In fact the more active the cell, the more the mitochondria increase in size and number by dividing.

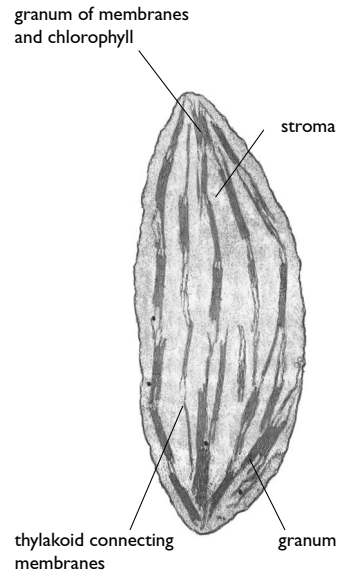
◆ CHECKPOINT SUMMARY

- ◆ The 'typical' animal cell under the electron microscope (EM) is the mammalian liver cell, as it contains a representative selection of those organelles found in animal cells
- ◆ The 'typical' plant cell under the EM is a leaf mesophyll cell
- ◆ The risk of distortion and the production of artefacts is greater with the EM as a result of the total dehydration, very thin sectioning, staining, and high temperatures generated
- ◆ It has been argued for example that the rough or granular endoplasmic reticulum is an artefact caused by the cracking and splitting of the cytoplasm pushing the ribosomes to the edge
- ◆ Mitochondria are rod-shaped organelles but appear oval in cross section. Chloroplasts are oval in structure and appear oval in cross section
- ◆ Lysosomes cannot be identified solely on appearance, the activity of digestive enzymes must be demonstrated by other means
- ◆ The rough and smooth endoplasmic reticula are not related in structure or function, and are not joined. Typically there is less smooth endoplasmic reticulum in plant cells
- ◆ Controversy surrounds the identification of centrioles in plant cells.



Chloroplasts

Chloroplasts contain the green pigment chlorophyll which absorbs light for photosynthesis. In photosynthesis light energy is used in the synthesis of organic compounds (e.g. glucose) from carbon dioxide and water. The energy is trapped in a stable form in the organic compounds. Chloroplasts, like mitochondria, have a double membrane, but there is also a third membrane system inside the chloroplast called the **thylakoid** membrane system. It is on this that the chlorophyll molecules are located. You can see from the diagram that the thylakoid membrane system is arranged in the form of stacked discs (**grana**) joined by connecting membranes (**lamellae**). The internal space is filled with a fluid (**stroma**) containing enzymes for making organic compounds, e.g. glucose and starch.



Cytoskeleton

An interconnected system of fibrous proteins gives cells their shape and a basis for movement. A number of thread like protein structures make up the cytoskeleton, notably actin, which forms thin contractile **microfilaments**; and tubulin, a protein which arranges itself into more rigid tubes resembling miniature scaffolding poles called **microtubules**. Microtubules form the supporting skeleton for the tiny cell 'hairs' called **cilia**. Microtubules are also formed and organised within areas called **centrosomes** (microtubule organising centres, or **MTOCs**) which lie very close to the nucleus. During cell division, the centrosome divides to form two new centres of microtubule formation at opposite sides of the cell. A net of microtubules (the spindle) forms between these two new centrosomes on which chromosomes are held and pulled apart.

In animal cells (but not all plant cells) the centrosome contains two structures, which can be seen in electron micrographs lying at right angles to each other. These are called **centrioles**. Centrioles also form the basal bodies at the base of cilia.

Plasma (cell surface) membrane

This controls the entry and exit of all materials into and out of cells. The electron microscope does not reveal any details of its structure, which is modelled on evidence of biochemical investigations. It is fatty in nature and contains proteins and other substances. It is partially permeable, being more permeable to water, than to dissolved solutes which pass through at different rates depending on a complex of factors.

In cells which are specialised for the exchange of materials across the plasma membrane e.g. cells lining the small intestine and parts of the kidney tubules, the surface area may be dramatically increased by microvilli. Microvilli are extensions of the plasma membrane which protrude out of the surface like the bristles of a hair brush.

Rough and smooth endoplasmic reticulum

In addition to the outer surface (plasma) membrane and those which surround the organelles, cells (especially secretory cells), have extensive internal membrane systems, known as the **endoplasmic reticulum** (ER). The ER is a maze of membrane bound tubules and

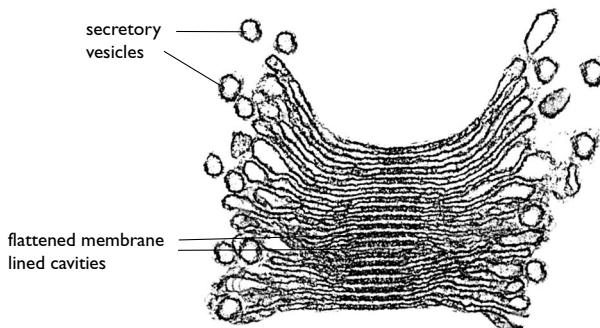
layers of flattened sacs enclosing a fluid filled interior, which acts as an intracellular (inside the cell) transport and storage system. It is continuous with the nuclear membrane and similar in structure.

The rough ER has ribosomes associated with it on the cytoplasmic side of the membranes. The ribosomes are the sites of protein synthesis.

The smooth ER tends to be more tubular, and appears to have a complex set of functions, including fat metabolism. The two types of ER are not connected.

Golgi apparatus

This is more clearly seen in animal cells and is a region of stacked smooth ER specialised for the purpose of collecting, processing and redirecting proteins and fats made in the rough ER. Materials are transported between the ER, the Golgi apparatus, and the plasma membrane, sealed in membrane sacs called vesicles which bud off from one membrane, and then fuse with another. Substances are taken in (**endocytosis**) and expelled (**exocytosis**) by the plasma membrane in this way. For example, digestive enzymes produced by cells of the pancreas are secreted by exocytosis into the pancreatic duct.



◆ CHECKPOINT SUMMARY

- ◆ Functions of organelles determined by differential centrifugation, and biochemical investigation of samples from each 'organelle band'
- ◆ Centrifuged 'organelle bands' not completely 'pure'
- ◆ Lysosomes poorly identifiable in electron micrographs, identification almost entirely on functional criteria
- ◆ Radioactive tracers can locate functions in specific organelles
- ◆ Cells specialised for particular functions show organelles developed in similar way, e.g. active voluntary muscle, and secretory cells, have an increased number of larger mitochondria.
- ◆ Smooth endoplasmic reticulum associated with a wide range of metabolic functions suggesting that its identification as a specific organelle is unlikely
- ◆ Existence of many ribosomes arranged in chains (polyribosomes) suggests protein synthesis (translation of mRNA) in action
- ◆ There are still major problems in correlating specific functions with clearly identifiable organelles
- ◆ Function of chloroplasts easily established with the use of the light microscope, i.e. recognising cell fraction that carries out photosynthesis.

Lysosomes

Vesicles produced by the Golgi apparatus are specialised for particular functions. Animal cells, have lysosomes which contain a cocktail of digesting enzymes. They break down and dispose of unwanted materials or damaged structures in the cell, expelling waste debris by exocytosis, and recycling reusable molecules within the cell. They also meet and fuse with vesicles carrying imported food substances, or immobilised bacteria, from the cell surface, and digest the contents.

It is clear from this that the membranes surrounding lysosomes must act as impermeable barriers preventing the leakage of its digestive enzymes into the cytoplasm, this process (**autodigestion**) occurs after the death of a cell and plays an important part in its removal.

The principal features of a bacterium

The features and organelles described above are typical of eukaryotic cells. Bacterial cells are **prokaryotic**. Their structure varies greatly to match the diversity of life style and habitat (see 10.1) and the illustration here is designed to show the major features which may be present, in a diagrammatic, generalised form. (Remember, no individual bacterium actually looks like this.)

Compare and contrast this structure with that of the eukaryotic cells. Most obviously, there are no large membrane-bound organelles. Instead of mitochondria and chloroplasts, the enzymes for respiration (and photosynthesis where this occurs) are located on simple membrane systems. In the absence of a nucleus, the DNA is formed, with its associated proteins, into a single main coil, the bacterial chromosome. Additional small loops of DNA called plasmids may also occur. Not all prokaryotes are able to move, but some possess flagella, which are like elongated cilia but without the microtubules, capable of propelling the organism through water. The cell wall is formed from a mixture of polysaccharides and amino acids called murein (not cellulose as in plants). Many bacteria which cause disease (pathogenic bacteria) have a slime capsule as their outermost layer which helps in protection against the body defences of the infected organism.

◆ CHECKPOINT SUMMARY

- ◆ Prokaryotic cells (bacteria) lack a true nucleus and membrane bound organelles
- ◆ The DNA is located in a central strand, and as circular plasmids in the rest of the cytoplasm
- ◆ The cell wall is not cellulose as in plant cells
- ◆ Respiration is located in mesosomes which are infoldings of the cell surface membrane, sharing the principle of increased surface area of membranes with true mitochondria
- ◆ The origin of mitochondria and chloroplasts as mutualistic prokaryotes with eukaryote cells, explains their absence in prokaryotes
- ◆ Ribosomes are present in both prokaryotes and eukaryotes
- ◆ Prokaryotic cells may form colonies but never multicellular organisms/tissues.

CELL FRACTIONATION

Ultracentrifugation to separate cell components

The function of individual organelles has been established largely by isolating each organelle from the rest of the cell components, and subjecting it to conditions which copy those which are thought to exist in the natural state. Naturally, the most detailed functional information has resulted from the organelles which are most easily isolated, namely mitochondria and chloroplasts. The isolation of subcellular components starts with a mechanical disruption of the cells to produce subcellular fragments, typically by breaking up fresh tissue in a test tube homogeniser. A pH buffer and sucrose solution are added to keep the cell fragments at optimum pH and ensure that the water potential of the surrounding liquid remains the same as organelles. Enzyme action and bacterial activity is slowed down by immersing tubes containing cell fragments in ice.

The fragments are separated by a series of spins in a high speed centrifuge (ultracentrifuge). The heaviest components (fractions) separate at the lowest spinning speeds. At speeds 1000 times the pull of gravity, the cell nuclei separate out into a pellet at the bottom of the tube. The remaining liquid (supernatant), containing all the other cell fragments may then be transferred to another tube and re-centrifuged at a higher speed. Mitochondria form a pellet at about 3000 times gravitational pull and the smaller cell fragments require even greater speeds. Isolated fragments are never pure, particularly the smaller components such as lysosomes because the cell membrane systems (ER, Golgi apparatus, nuclear and plasma membranes) reform into small vesicles of similar size when cells are broken up. Isolated cell fragments may be purified further by centrifugation in tubes which are prepared with concentrated gel-like solutes in the form of a density gradient so that the solution at the top of the tube is less dense than that at the bottom. As the tube is spun with the selected sample, so the cell fragments separate in bands at a level in the tube corresponding to their own density.

◆ CHECKPOINT SUMMARY

- ◆ Isolation of subcellular components starts with a mechanical disruption of the cells to produce subcellular fragments
- ◆ A pH buffer and sucrose solution is added to prevent disruption of the organelles, and the temperature is lowered to prevent autodigestion by enzymes, and decomposition by bacteria
- ◆ The suspension of cell fragments is spun at high speeds in an ultracentrifuge
- ◆ The force that is exerted upon the suspension forces the organelles downwards to the bottom of the tube according to their mass and density
- ◆ The nuclei are the first to be forced down at about 1000G (1000 x the force of gravity)
- ◆ The supernatant liquid can be separated off and respun at higher speeds
- ◆ Mitochondria spin down at about 3000G.
- ◆ Smaller organelles like the ribosomes require much higher forces to separate them out
- ◆ Isolated samples are never pure, and can be further purified by re-centrifuging in a liquid medium of graded density, with the greatest density at the bottom
- ◆ Organelles separate at a level in the liquid corresponding to their own density.

3

The Properties of plasma membranes related to the passage of substances through them

Contents

Plasma membranes

- ▼ The arrangement of phospholipids, proteins and carbohydrates in the fluid-mosaic model of membrane structure

Diffusion

- ▼ Diffusion and the factors which determine its rate. Fick's law

Water potential

- ▼ Osmosis - the movement of water from a solution of less negative water pressure to a solution of more negative water pressure through a partially permeable membrane

Active transport and facilitated diffusion

- ▼ The role of protein molecules in active transport and facilitated diffusion
- ▼ Endocytosis and exocytosis

PLASMA MEMBRANES

Introduction

The cell surface membrane (**plasmalemma** or **plasma membrane**) and the membranes which enclose the cell organelles and make up the extensive system of interrelated channels and vesicles within the cell share the same structure. It is a structure which is so flexible that it can break and reform easily yet it is capable of performing complex functions; acting as a barrier, container, transport regulator and as a site of recognition, distinguishing between 'friendly' and 'foreign' substances, 'self' and 'non-self'.

Membrane Structure - the fluid-mosaic model

Membranes are constructed from lipid, protein and carbohydrate components. The structure is based on a bilayer of phospholipid molecules, strengthened by cholesterol. Phospholipids adopt this bilayer structure automatically in fluid surroundings because the fatty acid 'tails' of the molecules are water repelling (hydrophobic) whilst the phosphate/alcohol 'heads' are water attracting (hydrophilic).

Proteins of various sizes and shapes are located on and in the bilayer. Some are bonded to the hydrophilic head region and do not penetrate the interior of the membrane (**extrinsic proteins**), others, have one end buried within the fatty layer and one end sticking

outside of the cell membrane (**intrinsic proteins**). A third kind, (**transmembrane proteins**), pass all the way through the membrane to create special channels. The carbohydrate component of cell membranes consists of short polysaccharide chains joined to membrane proteins or fats forming **glycoproteins** and **glycolipids** respectively, which project outside the cell membrane.

Individual molecules of phospholipid and protein in the membrane have a degree of **mobility** within the membrane. This fluidity, coupled with the bubbled appearance of the proteins on the surface of membranes gave rise to the term '**fluid mosaic model**' when the structure was first proposed. Cholesterol stabilises membranes by limiting the movement of phospholipid molecules within the membrane.

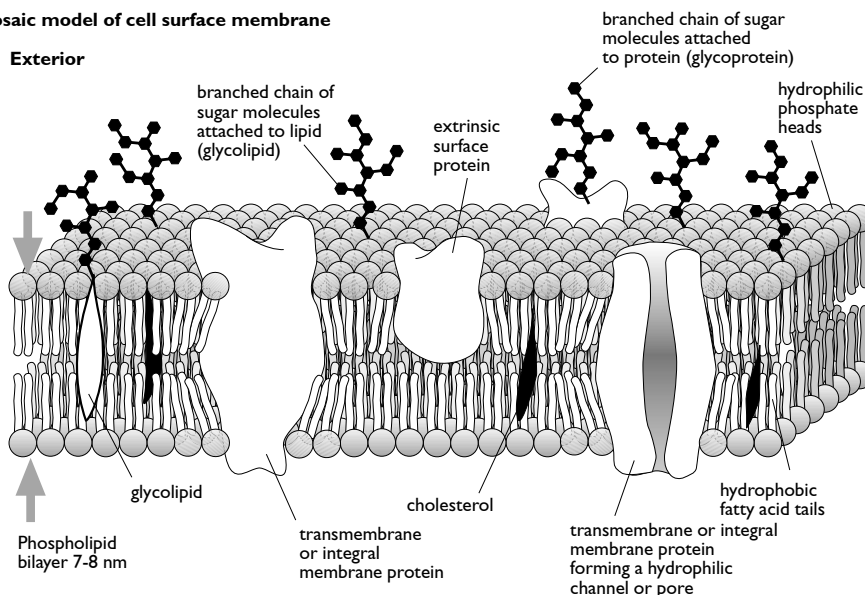
Cells have the ability to sense and respond to the presence of a great variety of molecules. This ability is due to proteins in the surface membrane which act as **receptors**. Hormones such as insulin and adrenaline, and many drugs and toxins lock onto recognition sites on receptor proteins triggering changes in the cell's behaviour.

Glycoproteins and glycolipids have a very important role in personalising a cell's identity. They are arranged in the membrane bilayer with the chain of sugar molecules to the outside. The possibility for variation in the structure and different combinations of these chains gives an endless range of different surface patterns, and forms the basis of a recognition system. Each individual has his or her own cell surface 'print'. 'Self' recognises 'self', and immune systems are mobilised when 'non-self' substances are encountered. The glycoproteins and glycolipids of the cell membrane are responsible for an individual's tissue type, a well known example of which is the blood groups.

◆ CHECKPOINT SUMMARY

- ◆ All cell membranes are thought to share the same structure except the inner membrane of mitochondria
- ◆ This is based on a lipid bilayer formed from phospholipids aligning themselves with their hydrophobic poles next to each other within the membrane, and hydrophilic poles facing outwards
- ◆ This fluid arrangement is stabilised by the presence of another lipid - cholesterol
- ◆ Various proteins are embedded within this lipid bilayer
- ◆ Extrinsic proteins are found on the surface of the membrane
- ◆ Intrinsic proteins have one end embedded in the lipid bilayer and the other protruding out of the external surface of the membrane
- ◆ Transmembrane proteins span the lipid bilayer and create aqueous channels of pores through which water soluble substances can pass by diffusion.
- ◆ Short polysaccharide chains attached to proteins (glycoproteins) and to fats (glycolipids) protrude from the external surface and act as cell recognition sites (receptors or antigens)
- ◆ The patchwork arrangement of all these types of molecules in the lipid bilayer is the origin of the description 'mosaic'.

Fluid mosaic model of cell surface membrane



Transport across cell membranes

The plasma membrane acts as a partially permeable barrier to the passage of substances into and out of the cell, for example water and fat soluble substances pass through more easily than sucrose. The different rate of passage of electrically charged ions generates membrane potentials which in turn influence the further uptake of charged ions, and are also involved in cell sensitivity, particularly in nerve cells.

The transport of respiratory gases, nutrients and metabolic waste products occurs through the membrane, either directly through the bilayer, or via channels or 'pores' created by transmembrane proteins. In some cases the substances move down a concentration gradient (i.e. from higher concentration to lower concentration) which is known as **passive transport**. In others, substances are moved against the concentration gradient (i.e. from lower concentration to higher concentration) in a process requiring energy in the form of ATP, which is known as **active transport**.

DIFFUSION and the factors which determine its rate

Molecules and ions may move passively by diffusion, which is defined as the overall (net) movement of substances from regions of their higher concentration (higher chemical potential) to regions of their lower concentration (lower chemical potential) until equilibrium is reached. The movement of the particles of substances in diffusion is random in all directions, as a result of their inherent kinetic energy. If there are more in one area than another, the net effect is for the particles to distribute themselves equally throughout that particular space, i.e. the concentration gradient evens out until at equilibrium there are an equal number of particles moving in all directions.

The speed of these diffusing particles is only increased by a rise in temperature, which increases their kinetic energy. In addition to the effect of temperature on diffusion, the amount of a substance that diffuses in a given time (rate of diffusion) is dependent upon the difference in its concentration in two separated regions (the **concentration gradient**), the surface area of the separating boundary or exchange surface, and the distance separating them i.e. the thickness of the exchange surface; a relationship expressed in the following equation known as **Fick's law**.

$$\text{Rate of diffusion} = \frac{\text{difference in concentration} \times \text{surface area}}{\text{thickness of the exchange surface}}$$

This gives a rough guide to the rate at which a substance such as oxygen diffuses from the air in an air sac (alveolus) in the lung into the blood in a lung capillary. In principle, the greater the surface area, the greater the concentration difference, and the thinner the separating layers, the greater the rate of diffusion.

The most important factor affecting diffusion across cell membranes, however, is the chemical nature of the substance being transported. Diffusion across the bilayer depends principally upon the solubility of the substance in fat. You will remember that the phospholipid bilayer is strongly hydrophobic. Polar molecules such as glucose, some amino acids, and inorganic ions pass through very slowly or not at all, whilst non polar molecules such as calciferol (vitamin D) move across with ease. It is interesting to note that the first anaesthetics (chloroform and ether) were fat solvents that disrupted membrane structure and function especially in nerve cells.

◆ CHECKPOINT SUMMARY

- ◆ The cell surface membrane defines the limits of the cell
- ◆ It receives 'messages' by providing receptor sites for hormones, cell recognition of 'self' and 'non-self', and act as antigens with which antibodies can bind
- ◆ It acts as a partially permeable membrane regulating the transport of substances across the membrane
- ◆ Internal membrane systems provide a surface for attachment of organelles and enzymes, and compartmentalise specialised functions within the cell.
- ◆ Passive diffusion occurs across membranes down diffusion gradients from high concentration to low concentration. Fat soluble substances diffuse faster than non-fat soluble substances which must pass through the aqueous pores
- ◆ The speed of these diffusing particles is only increased by an increase in temperature, which increases their kinetic energy. In addition to the effect of temperature on diffusion, the amount of a substance that diffuses in a given time (rate of diffusion) is dependent upon the difference in its concentration in two separated regions (the concentration gradient), the surface area of the separating boundary or exchange surface, and the distance separating them i.e. the thickness of the exchange surface; a relationship expressed by Fick's law: Rate of diffusion is proportional to the difference in concentration x surface area: divided by the thickness of the exchange surface.

WATER POTENTIAL

Osmosis

Cell membranes are **partially permeable**, allowing the passage of substances at different rates. Generally cell membranes are more permeable to water than to many solutes.

Water moves according to the same principles of diffusion, but it is not usual to refer to high and low 'concentrations' of water - the term 'concentration' being traditionally associated with any solutes dissolved **in** water in a solution. Therefore the preferred term is the chemical potential of water or **water potential**.

Water always moves (diffuses) from a region of higher water potential to one of lower water potential. You could think of water potential as the **tendency for water molecules to move** i.e. diffuse. The higher the water potential the more easily will the water molecules move, and the lower the water potential the less easily will the water molecules move.

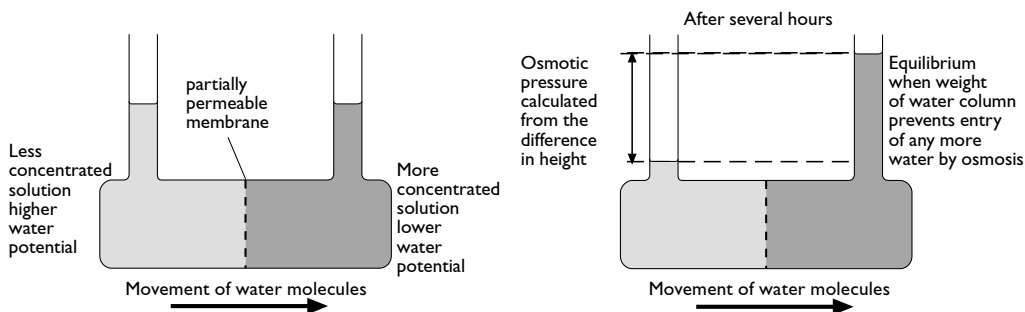
At standard pressure and temperature, pure water has the highest water potential. If substances are dissolved in solution then the water potential is decreased because the solutes associate with water to a greater or lesser extent and reduce the ease with which the water molecules can move. Therefore any solution has a lower water potential than pure water, and a more concentrated solution has a lower water potential than a less concentrated solution.

Water potential of a fluid can be described as the tendency of water to move into it from pure water. If the fluid **is** pure water, there is no movement, so the **water potential of pure water is zero**. The water potential of any solution is less than pure water and is therefore negative. The more negative the water potential the greater the tendency of water to move into that system.

Where a solution is separated by a partially permeable membrane from pure water or a less concentrated solution, water will diffuse into the more concentrated solution until equilibrium is reached. The special case of the diffusion of water across a partially permeable membrane is known as **osmosis**.

◆ CHECKPOINT SUMMARY

- ◆ Osmosis is the special case of the diffusion of water from regions of higher water potential to regions of lower water potential across a partially permeable membrane which is more permeable to water than to dissolved solutes. (If the membrane was fully permeable the solutes would diffuse down their diffusion gradient and the water would not move)
- ◆ At room temperature and pressure pure water has the highest water potential which is given the value of zero. Solutions have a water potential of less than zero (negative)
- ◆ Facilitated diffusion occurs via specialised carriers in the membranes which reduce the resistance to the diffusion of these substances
- ◆ Active transport involves energy in the form of ATP from respiration to pump ions across the cell surface membrane via carriers against their diffusion gradient
- ◆ Mass or bulk transport of fluids into and out of the cell is achieved by vacuoles fusing with, or being pinched off from, the cell surface membrane respectively.



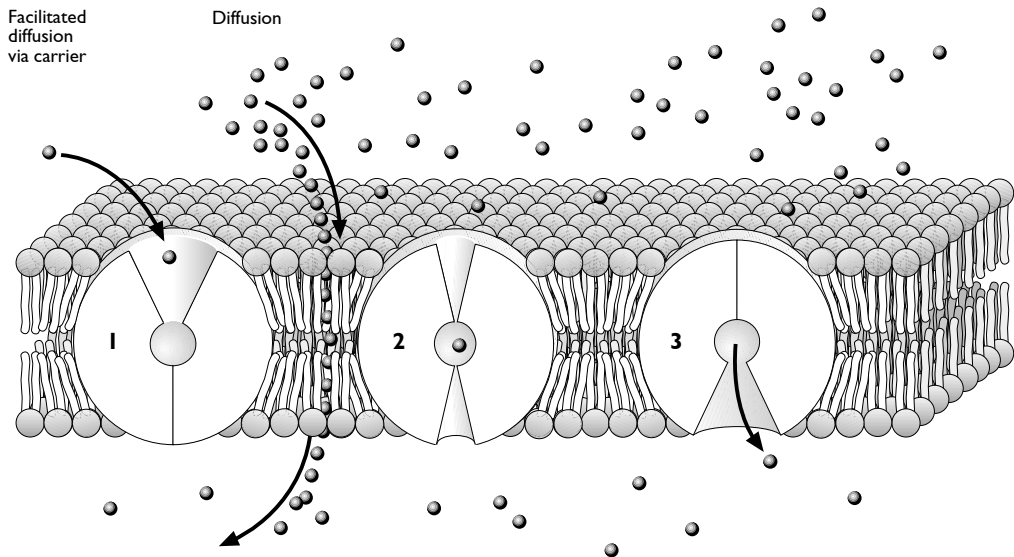
FACILITATED DIFFUSION

The transport of polar molecules e.g. glucose, some amino acids, and inorganic ions, across cell membranes, occurs through hydrophilic channels formed by transmembrane proteins, in a process called **facilitated diffusion**. Polar molecules also become temporarily attached to special binding sites on transmembrane proteins called **carriers**. These are rather like enzymes in that they are shape specific and accelerate a process which is already 'downhill' in terms of energy. All that is needed is a concentration gradient. The attachment of a molecule to the binding site alters the overall shape of the carrier in such a way as to deposit the molecule on the other side of the membrane along their normal concentration gradients.

As has been seen, the transport of inorganic ions such as sodium (Na^+), potassium (K^+), calcium (Ca^{++}) and chloride (Cl^-) across cell membranes depends upon transmembrane proteins.

Some transmembrane proteins form selective **ion channels** through which the facilitated diffusion of ions occurs down their concentration gradients. These ion channels cannot be coupled to an energy source, and cannot be involved in active transport across the membrane.

Diagram to show diffusion and facilitated diffusion



ACTIVE TRANSPORT

Some transmembrane proteins act as active **ion pumps**, which use energy (in the form of ATP) from respiration, to move ions against their concentration gradients. The ion channels can open and close like 'gates', and the activity of the ion 'pumps' can be varied. In these ways, the membrane can be made selectively permeable to certain ions at certain times.

As a result of the distribution of ions on either side of the membrane **transmembrane potentials** are created, in which there is an overall difference in electric charge between the inside and the outside of the membrane. In normal conditions, the inside of the cell membrane is negatively charged relative to the outside, and this will assist the uptake of positively charged ions (cations) and oppose the uptake of negatively charged ions (anions). Thus the movement of ions across membranes is influenced by both electrical and chemical gradients i.e. **electro-chemical gradients** (these have particular importance for the function of nerve and muscle cells).

Some of these transmembrane protein carriers carry two substances at once in a **coupled mechanism**. A common example is the sodium/potassium pump (Na^+/K^+ pump), in which the active pumping of sodium out of a cell is coupled with the pumping-in of potassium, both against their diffusion gradients (for every two sodium ions pumped out, three potassium ions are pumped in, resulting in high potassium and low sodium levels within the cell). Another example is where the sodium pump is linked to the uptake of glucose. The sodium ions are pumped actively out of the cell and diffuse back into the cell via a carrier associated with glucose. In this case the glucose is moving passively down its concentration gradient, but at a faster rate as a result of its association with the re-entry of sodium which is kept at a high rate by its being continually pumped out of the cell.

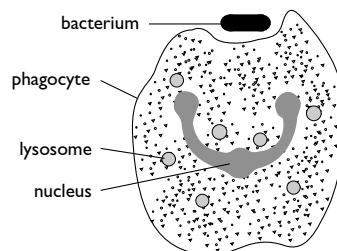
Endo and exocytosis

The bulk transport of liquids and solids is achieved by membranes pinching off small bubble-like vesicles which can fuse with other membranes to transfer their contents. Uptake of substances into the cell in this way is known as **endocytosis**, and elimination of substances as **exocytosis**.

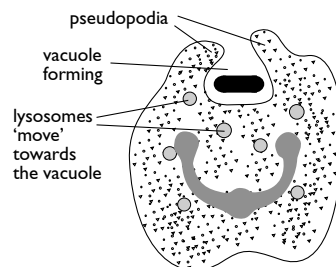
The term **phagocytosis** is used to describe the kind of endocytosis in which relatively large solid particles are 'ingested' by a cell, as in the case of white blood cells which engulf bacteria or *Amoeba* taking in food. In this process the outer membrane folds inwards to completely surround the ingested particle. It is then transported into the interior of the cell within a sealed vesicle which functions as a temporary digestive chamber and, in *Amoeba*, is termed a food vacuole. Lysosomes containing digestive enzymes fuse with and release their contents into the food vacuole and the soluble products of digestion are transported out for assimilation by the cell. Fluids may be taken up in the same way. This process is sometimes referred to as **pinocytosis**.

Undigested matter may be expelled from the cell by the reverse process (exocytosis), similarly fluid secretions produced by cells are transported to the plasma membrane in small vesicles, which fuse with it and release their contents.

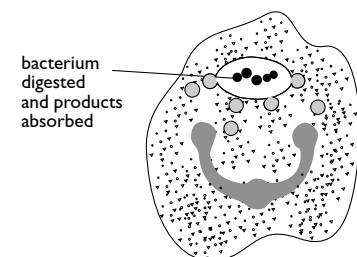
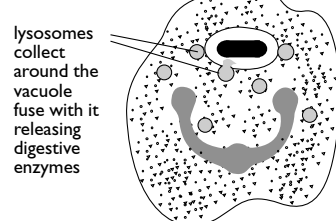
Phagocyte 'moves' towards bacterium



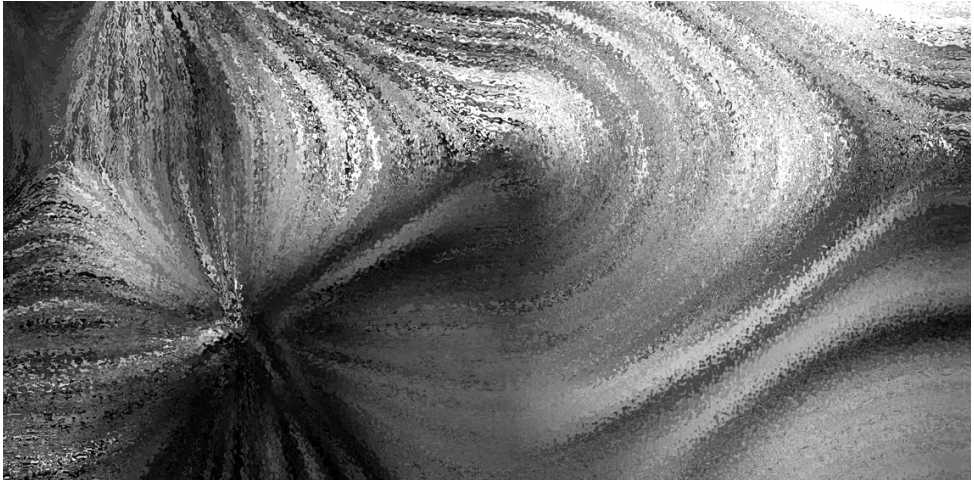
Vacuole forming



Vacuole formed



Topic Guides



- 1 The cell as the basic unit of structure in prokaryotic and eukaryotic organisms**
- 2 The electron microscope and the technique of cell fractionation to study ultrastructure**
- 3 The properties of plasma membranes and the passage of substances through them**

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Topic Guide T

Session: _____

KEY SKILLS N3 all**1 The cell as the basic unit of structure****I The cell as the basic unit of structure in prokaryotic and eukaryotic organisms**

Prokaryotic cells

Check List

- ▼ Prokaryotic cells (bacteria) lack a true nucleus and membrane bound organelles.
- ▼ The DNA is located in a central strand, and as circular plasmids in the rest of the cytoplasm.
- ▼ The cell wall is not cellulose as in plant cells.
- ▼ Respiration is located in mesosomes which are infoldings of the cell surface membrane, sharing the principle of increased surface area of membranes with true mitochondria.
- ▼ The origin of mitochondria and chloroplasts as mutualistic prokaryotes with eukaryote cells, explains their absence in prokaryotes.
- ▼ Ribosomes present in both.
- ▼ Prokaryotic cells may form colonies but never multicellular organisms/tissues.

Student Activity**Materials**

- Microscopes
- Slides of bacteria, and of squamous epithelium
- OHT Prokaryotic cell

Procedure

Observe the two slides under the microscope.

Make a drawing of the squamous epithelium cells and then draw the bacteria as seen down the light microscope ON the drawing of the squamous epithelium cells

Comment on their relative sizes and relate this to the fact that many bacteria are disease causing (pathogenic).

Compare what you have seen down the microscope with the □ OHT Prokaryotic cell, and comment on the relative resolution and magnification of the electron microscope when compared to your light microscope.

Set work

Much of the original work on the function of DNA was done on prokaryotic cells, write a paragraph suggesting difficulties in extrapolating the findings of this work to eukaryotic cells.

Notes:

Topic Guide

Session: _____

KEY SKILLS

1 The cell as the basic unit of structure

1 The cell as the basic unit of structure in prokaryotic and eukaryotic organisms

Eukaryotic cells

Check List

- ▼ Eukaryotes do have a true membrane bound nucleus containing chromosomes of DNA and histone proteins, and membrane bound organelles as described previously.
- ▼ Eukaryotic cells typically form tissues in multicellular organisms.
- ▼ Cell differentiation and specialisation prevent the easy definition of typical animal and plant cells.
- ▼ 'Typical' cells would be those considered to show features common to the vast majority of cells in animals or plants
- ▼ A squamous (pavement) epithelium cell from the lining of the mouth could be considered as a 'typical' animal cell.
- ▼ A mesophyll cell from a leaf could be considered as a 'typical' plant cell.
- ▼ Under the best light microscope both the typical animal cell and the typical plant cell can be seen to possess a cell surface membrane, cytoplasm, nucleus with nucleolus, mitochondria, endoplasmic reticulum, and Golgi body.
- ▼ In addition the typical plant cell can be seen to possess a cell wall around the cell surface membrane, a large central vacuole surrounded by the tonoplast membrane, and chloroplasts.

Student Activity

Materials

- Microscopes
- Slides of squamous epithelium
- Slides TS Leaf showing palisade cells
- OHT Photomicrograph of animal cells
- OHT Photomicrograph of plant cells

Procedure

Make LP and HP drawings of the plant and animal cells. Using the OHTs for guidance.

Note the major differences:

- a) in what is actually seen;
- b) what is known from theory studies.

Explain the reasons for differences between (a) and (b)

Set Work

Write a brief justification of 'interpretation' in Biological drawing, e.g. drawing the plant cell wall as a double line (which is not actually seen down the typical lab microscope).

Notes:

Topic Guide

Session: _____

KEY SKILLS N3 all

1 The cell as the basic unit of structure

1 The cell as the basic unit of structure in prokaryotic and eukaryotic organisms

Eukaryotic cells

Check List

- ▼ If the specimen can be observed with the naked eye without the need for magnification the calculation of the linear magnification of any drawing of it is straightforward - being the number of times any linear dimension of the drawing is greater than that of the specimen.
- ▼ Magnifications of images of slides of biological material are often given in terms relating to the magnification of the microscope image, e.g. $\times 100$ and $\times 400$.
- ▼ However reproductions of the image as seen down a microscope introduce another layer of magnification which is often overlooked.
- ▼ To calculate the linear magnification of drawings of microscope images of specimens it is necessary to know how many times the drawing is larger than the actual specimen.
- ▼ The actual size of the specimen is measured with an eye piece graticule and stage micrometer.

Student Activity

Materials

- Microscopes
- Material for the preparation of temporary mounts
- Slides of TS Leaf
- Practical Work Sheet:
The preparation of temporary mounts, the use of simple staining techniques and the estimation of size
- ☐ The use of graticule and stage micrometers

Procedure

- ☐ OHT aids understanding of the use of an eye piece graticule and stage micrometer.

Work out the linear magnification of a drawing that you make of a structure seen under the microscope. Explain all your steps fully.

Set work

Calculate the magnification of the area of a circle with a diameter of 2 cm, when the diameter is doubled (linear magnification of $\times 2$), and trebled (linear magnification of $\times 3$).

Try to work out why microscopic animals appear to move so fast when seen moving under the microscope.

Notes: _____

AS Module 1

Molecules, Cells & Systems

2 The electron microscope and the technique of cell fractionation to study ultrastructure

Electron microscopy

Topic Guide

Session:

KEY SKILLS

Check List

- ▼ Electron microscopes use a beam of electrons, instead of light. The electrons are focussed by magnets onto a fluorescent screen to produce an image which may be photographed to produce electronmicrographs.
- ▼ Electron beams have a much shorter wavelength than light which reveals much more detail of the specimen, and allows useful magnification up to x500 000.
- ▼ Specimens must be completely dehydrated and mounted in a vacuum to avoid scattering of the electrons
- ▼ There are two types of electron microscope, the transmission electron microscope (TEM) and the scanning electron microscope (SEM).
- ▼ In the TEM, electrons pass through very thin sections which have been stained with salts of heavy metals such as lead and uranium to enhance differentiation between structures
- ▼ In the SEM, electrons are reflected off of the surfaces of specimens to give 3D images.
- ▼ The major limitation of the use of electron microscopy is that the picture of the specimen is far removed from the reality of the living tissue or organism.
- ▼ The drastic preparation of the specimen, including complete dehydration, very thin sectioning, staining, high temperatures and vacuum conditions, raises problems of the interpretation of the image, and the production of 'artificial' structures (artefacts).

Student Activity

Materials

- Transmission electronmicrographs
- Scanning electronmicrographs
- OHT Light micrograph
- OHT Electron micrograph

Procedure

Examine the TEMs and the SEMs concentrating on magnification and resolution.

Consider the problem why the sac-like cisternae of the rough endoplasmic reticulum are almost always seen in cross section only.

Try to counter the argument that the rough endoplasmic reticulum is in fact an artefact caused by the splitting of the thin, dehydrated, heated cytoplasm, pushing ribosomes to the edge of the cracks where they appear to line up on a membrane.

Set work

Write a few lines explaining why the artificial colouring of electronmicrographs is misleading.

Notes:

AS Module 1

Molecules, Cells & Systems

2 The electron microscope and the technique of cell fractionation may be used to study ultrastructure

Electron microscopy

Topic Guide

Session: _____

KEY SKILLS N3 all

Check List

- ▼ Resolution is the ability to see detail.
- ▼ The higher the resolution the more of the detail present can be seen.
- ▼ Magnification is enlarging in size.
- ▼ Increasing magnification only reveals extra detail if the detail can be resolved.
- ▼ Resolving power of light microscope limited by the wavelength of light.
- ▼ Put simply some detail is so small that it cannot be 'hit' by light, and therefore cannot be seen.
- ▼ Endless magnification of the image produced by a light microscope yields no extra information.
- ▼ Wavelengths of electron beams much shorter than that of light and give the electron microscope much greater resolving power than the light microscope.
- ▼ Therefore extra magnification up to $\times 250\,000$ yields an equivalent amount of detail.

Student Activity

Materials

- Microscope
- Prepared slide of TS dicotyledonous leaf
- OHT Magnification and Resolution.
- OHT Electron micrograph

Procedure

Set up slide of TS Leaf showing xylem on high power

Although this may be the first sight of TS dicotyledonous leaf, it can clearly be seen to be made up of cells.

Even under the highest power ($\times 400$) very little detail will be seen within the cells.

- OHT Magnification and Resolution.
- OHT Electron micrograph

Measure the size (diameter) of a mitochondrion on the electron micrograph and calculate the actual size by dividing by the magnification.

Set work

1. Distinguish between the terms 'magnification' and 'resolution'.
2. Why can more detail be seen using a beam of electrons than using visible light?

Notes:

AS Module 1

Molecules, Cells & Systems

2 The electron microscope and the technique of cell fractionation may be used to study ultrastructure

Cell ultrastructure

Topic Guide

Session: _____

KEY SKILLS C3.1a: C3.1b

Check List

- ▼ The 'typical' animal cell under the electron microscope (EM) is the mammalian liver cell, as it contains a representative selection of those organelles found in animal cells.
- ▼ The 'typical' plant cell under the EM is a leaf mesophyll cell.
- ▼ The risk of distortion and the production of artefacts is greater with the EM as a result of the total dehydration, very thin sectioning, staining, and high temperatures generated.
- ▼ It has been argued for example that the rough or granular endoplasmic reticulum is an artefact caused by the cracking and splitting of the cytoplasm pushing the ribosomes to the edge.
- ▼ Mitochondria are rod-shaped organelles but appear oval in cross section. Chloroplasts are oval in structure and appear oval in cross section
- ▼ Lysosomes cannot be identified solely on appearance, the activity of digestive enzymes must be demonstrated by other means.
- ▼ The rough and smooth endoplasmic reticula are not related in structure or function, and are not joined. Typically there is less smooth endoplasmic reticulum in plant cells.
- ▼ Controversy surrounds the identification of centrioles in plant cells.

Student Activity

Materials

- A selection of e.m.s showing whole cells or parts of cells, both animal and plant.
- T.E.M. image of a whole cell (unlabelled, for homework)
- OHT Ultrasound scan of fetus in uterus

Procedure

- OHT Ultrasound scan of fetus in uterus demonstrates that something imaged in an unfamiliar way requires practice to interpret.

Examine the electronmicrographs.

Is it plant or animal? Look at the junction between adjacent cells. Is there a cell wall? Any large spaces?. Look to identify the larger things first, e.g. nucleus, mitochondria, chloroplasts.

Discuss clues which point to particular functions e.g. lots of mitochondria, cilia, microvilli etc.

Set work

Label the T.E.M. of an animal cell before the next lesson.

Notes:

AS Module 1

Molecules, Cells & Systems

2 The electron microscope and the technique of cell fractionation may be used to study ultrastructure

Cell ultrastructure

Topic Guide

Session:

KEY SKILLS C3.1a: C3.1b
IT3.1: IT3.3

Check List

- ▼ Functions of organelles determined by differential centrifugation, and biochemical investigation of samples from each 'organelle band'.
- ▼ Centrifuged 'organelle bands' not completely 'pure'.
- ▼ Lysosomes poorly identifiable in electron micrographs, identification almost entirely on functional criteria.
- ▼ Radioactive tracers can locate functions in specific organelles.
- ▼ Cells specialised for particular functions show organelles developed in similar way, e.g. active voluntary muscle, and secretory cells, have increased number of larger mitochondria.
- ▼ Smooth endoplasmic reticulum associated with a wide range of metabolic functions suggesting that its identification as a specific organelle is unlikely.
- ▼ Existence of many ribosomes arranged in chains (polyribosomes) suggests protein synthesis (translation of mRNA) in action.
- ▼ There are still major problems in correlating specific functions with clearly identifiable organelles.
- ▼ Function of chloroplasts easily established with the use of the light microscope, i.e. recognising cell fraction that carries out photosynthesis.

Student Activity

Materials

- A selection of electronmicrographs showing whole cells or parts of cells, both animal and plant.
- T.E.M. image of a whole cell

Procedure

Revise the identification of all organelles and membrane systems.

Construct a table of organelles and their function.

Set work

Explain the structure and function of a mitochondrion in relation to its surface area to volume ratio.

Notes:

AS Module 1

Molecules, Cells & Systems

2 The electron microscope and the technique of cell fractionation may be used to study ultrastructure

Cell ultrastructure

Topic Guide

Session: _____

KEY SKILLS N3 all

Check List

- ▼ Prokaryotic cells (bacteria) lack a true nucleus and membrane bound organelles.
- ▼ The DNA is located in a central strand, and as circular plasmids in the rest of the cytoplasm.
- ▼ The cell wall is not cellulose as in plant cells.
- ▼ Respiration is located in mesosomes which are infoldings of the cell surface membrane, sharing the principle of increased surface area of membranes with true mitochondria.
- ▼ The origin of mitochondria and chloroplasts as mutualistic prokaryotes with eukaryote cells, explains their absence in prokaryotes.
- ▼ Ribosomes are present in both prokaryotes and eukaryotes.
- ▼ Prokaryotic cells may form colonies but never multicellular organisms/tissues.

Student Activity

Materials

- Microscope and slides of bacteria

□ OHT Structure of bacterium

Procedure

Observe the prepared slides of bacteria, and calculate the size, either by approximation or the use of the stage micrometer and eye piece graticule.

Observe □ OHT Structure of bacterium and estimate the magnification needed to reveal such detail.

Set work

Explain the relationship between the structure of an individual bacterium and the type of colony that they form.

Notes:

AS Module 1

Molecules, Cells & Systems

2 The electron microscope and the technique of cell fractionation may be used to study ultrastructure

Cell fractionation

Topic Guide

Session: _____

KEY SKILLS

Check List

- ▼ Isolation of subcellular components starts with a mechanical disruption of the cells to produce subcellular fragments.
- ▼ A pH buffer and sucrose solution is added to prevent disruption of the organelles, and the temperature is lowered to prevent autodigestion by enzymes, and decomposition by bacteria.
- ▼ The suspension of cell fragments is spun at high speeds in an ultracentrifuge.
- ▼ The force that is exerted upon the suspension forces the organelles downwards to the bottom of the tube according to their mass and density.
- ▼ The nuclei are the first to be forced down at about 1000G (1000 x the force of gravity).
- ▼ The supernatant liquid can be separated off and respun at higher speeds.
- ▼ Mitochondria spin down at about 3000G.
- ▼ Smaller organelles like the ribosomes require much higher forces to separate them out.
- ▼ Isolated samples are never pure, and can be further purified by recentrifuging in a liquid medium of graded density, with the greatest density at the bottom.
- ▼ Organelles separate at a level in the liquid corresponding to their own density.

Student Activity

Materials

- Centrifuge
- Blender
- Ice
- Iodine in potassium iodide solution
- Microscope and slides

Procedure

Macerate some leaves (spinach) in ice cold 0.5 M sucrose in the blender, filter through some muslin, and spin at low speed in the centrifuge for 10 minutes. This should separate down cell walls and starch grains.

Pour off supernatant and re-centrifuge at high speed for 10 minutes to separate out chloroplasts.

Observe samples of the fractions under the light microscope.

Stain both fractions with iodine in potassium iodide solution to test for the presence of starch.

Set work

Write a short account of your findings.

Notes:

AS Module 1

Molecules, Cells & Systems

3 The properties of plasma membranes and the passage of substances through them

Plasma membranes

Topic Guide

Session: _____

KEY SKILLS

Check List

- ▼ All cell membranes are thought to share the same structure except the inner membrane of mitochondria.
- ▼ This is based on a lipid bilayer formed from phospholipids aligning themselves with their hydrophobic poles next to each other within the membrane, and hydrophilic poles facing outwards.
- ▼ This fluid arrangement is stabilised by the presence of another lipid - cholesterol.
- ▼ Various proteins are embedded within this lipid bilayer.
- ▼ Extrinsic proteins are found on the surface of the membrane.
- ▼ Intrinsic proteins have one end embedded in the lipid bilayer and the other protruding out of the external surface of the membrane.
- ▼ Transmembrane proteins span the lipid bilayer and create aqueous channels of pores through which water soluble substances can pass by diffusion.
- ▼ Short polysaccharide chains attached to proteins (glycoproteins) and to fats (glycolipids) protrude from the external surface and act as cell recognition sites (receptors or antigens).
- ▼ The patchwork arrangement of all these types of molecules in the lipid bilayer is the origin of the description 'mosaic'.

Student Activity

Materials

- ☐ OHT Phospholipids and the bilayer
- ☐ OHT The fluid mosaic model

Procedure

☐ OHT Phospholipids and the bilayer explains the structure of cell membranes, building up the fluid mosaic model from the phospholipid bilayer. Use this OHT as a back transparency for drawing in first cholesterol (slows down lateral movement of phospholipids and also makes the membrane less permeable), then the three types of protein, extrinsic, intrinsic, and transmembrane. Finally draw in glycoproteins and glycolipids.

☐ OHT summarises the final structure.

Place some well washed identical slices of beetroot (washed until no more pigment is released) in beakers of water at a range of temperatures from 0°C to 100°C; and in a range of dilutions of detergent, and observe the release of pigment from the slices. Use a colorimeter if available.

Set work

List all the structures in plant, animal and bacterial cells which include membranes in their structure.

Tabulate the results from the experiment with beetroot slices, and comment on the results.

Notes:

AS Module 1

Molecules, Cells & Systems

3 The properties of plasma membranes and the passage of substances through them

Diffusion

Topic Guide

Session: _____

KEY SKILLS C3.3

Check List

- ▼ The cell surface membrane defines the limits of the cell.
 - ▼ It receives 'messages' by providing receptor sites for hormones, cell recognition of 'self' and 'non-self', and act as antigens with which antibodies can bind.
 - ▼ It acts as a partially permeable membrane regulating the transport of substances across the membrane.
 - ▼ Internal membrane systems provide a surface for attachment of organelles and enzymes, and compartmentalise specialised functions within the cell.
 - ▼ Passive diffusion occurs across membranes down diffusion gradients from high concentration to low concentration. Fat soluble substances diffuse faster than non-fat soluble substances which must pass through the aqueous pores.
 - ▼ The speed of these diffusing particles is only increased by an increase in temperature, which increases their kinetic energy. In addition to the effect of temperature on diffusion, the amount of a substance that diffuses in a given time (rate of diffusion) is dependent upon the difference in its concentration in two separated regions (the concentration gradient), the surface area of the separating boundary or exchange surface, and the distance separating them i.e. the thickness of the exchange surface; a relationship expressed by Fick's law.
- Rate of diffusion is proportional to:
- difference in concentration x surface area: divided by the thickness of the exchange surface

Student Activity

Materials

- Gelatine and methylene blue
- 'Jelly' mould or equivalent
- OHT Diffusion and facilitated diffusion

Procedure

Make up different sized cubes of gelatine coloured with methylene blue, wash well to remove any surface dye, and submerge in clear water.

Observe the diffusion of dye from the cubes.

Use a colorimeter or colour chart to try to quantify rate of diffusion of dye out of cubes with respect to surface area to volume ratio.

Set work

Use an example from the human body to illustrate Fick's law.

Notes:

AS Module 1

Molecules, Cells & Systems

3 The properties of plasma membranes and the passage of substances through them

Water potential

Active transport and facilitated diffusion

Endo- and exocytosis

Topic Guide

Session:

KEY SKILLS C3.3

Check List

- ▼ Osmosis is the special case of the diffusion of water from regions of higher water potential to regions of lower water potential across a partially permeable membrane which is more permeable to water than to dissolved solutes. (If the membrane was fully permeable the solutes would diffuse down their diffusion gradient and the water would not move.)
- ▼ At room temperature and pressure pure water has the highest water potential which is given the value of zero. Solutions have a water potential of less than zero (negative).
- ▼ Facilitated diffusion occurs via specialised carriers in the membranes which reduce the resistance to the diffusion of these substances.
- ▼ Active transport involves energy in the form of ATP from respiration to pump ions across the cell surface membrane via carriers against their diffusion gradient.
- ▼ Mass or bulk transport of fluids into and out of the cell is achieved by vacuoles fusing with, or being pinched off from, the cell surface membrane respectively.

Student Activity

Materials

- Range of plant food storage material
- 1M sucrose solution
- Methylene blue
- Practical Work Sheet:
The movement of water down water potential gradients through partially permeable membranes.

☐ OHT Water potential

Procedure

Investigate the water potential of a range of plant material: potato chips, beetroot (which allows for a check on any losses from damaged cells), onion rings, banana slices of different ripeness.

Measure changes in dimension and/or mass.

An alternative approach is to investigate any alterations of density of bathing solution by colouring with methylene blue and releasing a drop of this carefully at mid-depth into a beaker of the original solution, if water has entered the cells by osmosis the bathing solution will have become more concentrated, and will sink when introduced into original solution. If the bathing solution has gained water from the cells it will be less dense than the original and rise when introduced into the original.

Osmosis and diffusion can be studied in the same investigation if solutions of urea are used. Initially plant material loses water to concentrated urea solution and shrinks and loses mass, but after a

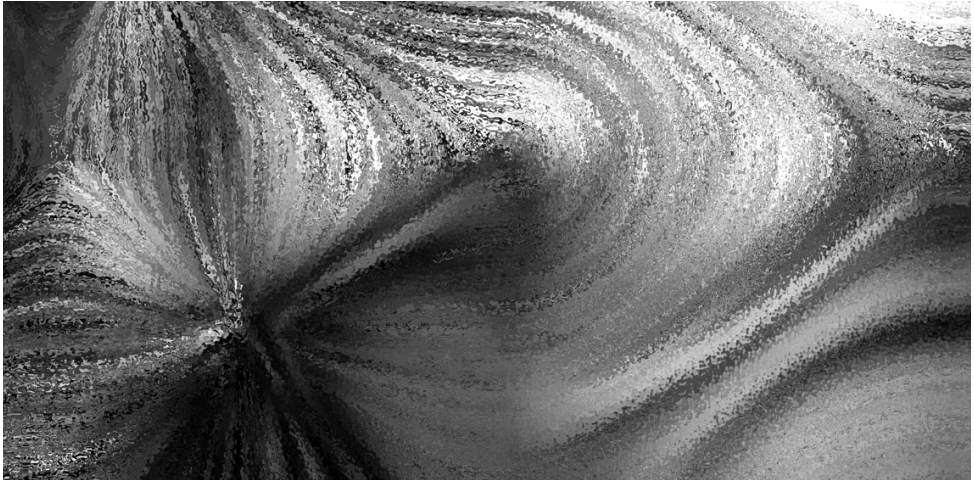
period urea diffuses into the plant material which recovers size and mass. A puzzling and interesting phenomenon to observe.

Set work

Comment critically on the the relative merits of using changes in dimensions or mass to detect losses and gains of water.

Notes:

Practical Guides



- 1 The cell as the basic unit of structure in prokaryotic and eukaryotic organisms**
- 3 The properties of plasma membranes and the passage of substances through them**

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PRACTICAL GUIDE

Cells

I The cell as the basic unit of structure in prokaryotic and eukaryotic organisms

Eukaryotic cells

Practical work to include the preparation of temporary mounts and the use of simple staining techniques.

The preparation of temporary mounts, the use of simple staining techniques

Introduction

Plant and animal cells are to be studied under the light microscope. The plant cells are to be viewed by making a temporary preparation of the inner epidermis of an onion scale, stained with iodine in potassium iodide solution to make the cells more visible. A prepared slide of human epithelial cells from the lining of the cheek will also be studied.

Method

- ▼ Place 1-2 drops of iodine in KI solution in the middle of the slide.
- ▼ Take a piece of onion scale and tear it with the fingers. This should leave a very thin epidermis attached to the inside surface of the scale. Carefully remove a small piece (about 0.5 cm²) of this epidermis with the forceps, and place it in the drop of iodine solution on the slide.
- ▼ Use the mounted needle to position the piece of epidermis flat in the iodine solution, then use the needle again to lower the coverslip carefully onto the specimen, (trying not to trap too many air bubbles).
- ▼ Examine the slide under the light microscope, using the medium power (x10) objective lens, and draw 2-3 adjacent cells (at least a few cm across). Label the drawing to show the structures you can see, e.g. nucleus, cell wall, vacuole, and cytoplasm (they may not all be visible). Annotate the drawing to show the functions of the structures you have drawn.
- ▼ Calculate the magnification of the drawing:

$$\text{Microscope magnification} = \text{eyepiece lens magnification} \times \text{objective lens magnification}$$

Note this figure on your drawing.

- ▼ Now correct the magnification for the size of the drawing; that is the number of times the drawing is larger than the image seen down the microscope eg, twice the size [x2]. This gives a final magnification of:

$$\text{Eyepiece lens} \times \text{objective lens} \times 2$$

Also note this figure on your drawing.

- ▼ Use the high power (x40) objective lens to check the details you have drawn.
- ▼ Now examine the prepared slide of human cheek cells under the microscope. Locate the cells using the medium power (x10) objective lens, you will probably need a higher power to make the drawing.
- ▼ Draw 2-3 cells (not too small), label the drawing, annotate, and note the final magnification (microscope magnification x size difference).
- ▼ Make sure the drawings have suitable headings.

Teaching Notes

This practical usually works well, provided the student gets a clean piece of the onion epidermis, without any additional tissue. They also need to make sure it is flat under the coverslip, and hopefully without too many air bubbles.

The student can usually see the vacuole and cell wall clearly. They may see the nucleus, which is usually pushed over to one side of the cell by the vacuole. Occasionally it is possible to see the cell membrane in the corners, if the cells have plasmolysed slightly. Cytoplasm is usually visible as a granular material in the corners of the cells.

The cheek cells have less detail, although the nucleus can be clearly seen. Some cells may show granules in the cytoplasm.

This practical also provides an opportunity to discuss the main points of **biological drawing technique**. The important points to get across are:

Drawings should be line drawings, with no shading, or use of colour.

They should be large enough to be clear.

The lines should not be sketchy.

Pencils should be sharp, HB or H.

Label lines should be drawn with a ruler, and should not cross. The labels should be written in pencil, and should be well away from the drawing.

Labels should be annotated, either next to the label or as a suitable note, to give further information as required.

Magnification, or an indication of size/scale, should be included.

Drawings should be clearly labelled with the student's name, the name of the specimen, its classification if appropriate, type of section/squash etc, and preparation or staining details.

Sometimes, depending on the microscope and the slide, the plant cell wall cannot be clearly seen to have a thickness, but it is always drawn with a double line to distinguish it from membranes.

Requirements per student

- Microscopes
- Slide and coverslip
- Forceps
- Mounted needle
- Drawing materials
- Iodine in potassium iodide solution
- Pieces of fresh onion scale
- Prepared slide of Human cheek cells

Safety Considerations

Iodine in potassium iodide solution can be an irritant, and will stain clothes if spilled. Microscopes with adjustable mirrors must not be used in direct sunlight.

AS Module 1 (Biology and Human Biology) Molecules, Cells and Systems

I The cell as the basic unit of structure in prokaryotic and eukaryotic organisms

Eukaryotic cells

Practical work to include the estimation of size.

The estimation of size of microscopic structures

Introduction

There are two methods you can use to measure the size of objects under the light microscope. One is a calculation based on the magnification, the other is to use an eyepiece graticule, which will need to be calibrated for the microscope using a stage micrometer. The graticule will give you a more accurate measurement, but the procedure is more complex.

Method A - Using the magnification

Make a note of the magnification of the eyepiece lens, and of the objective lens. The total magnification is found thus:

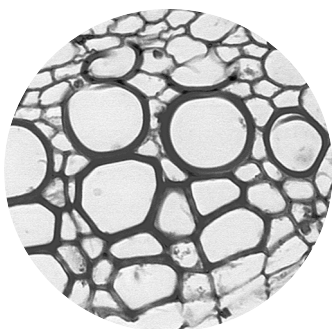
$$\text{Total magnification} = \text{magnification of eyepiece lens} \times \text{magnification of objective lens}$$

The term **linear magnification** refers to the number of times an imaginary 'line' across the drawing is greater than the equivalent would be on the original specimen (as opposed, for example, to considering the magnification of the area).

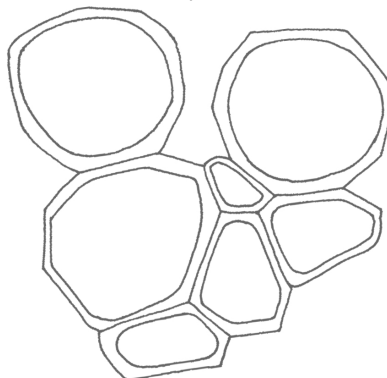
To calculate the linear magnification of the drawing, estimate how large the drawing is, compared with the view down the microscope. Look down the microscope, and then at the drawing, is the drawing x2, x3, x4 the size of the specimen?

You can then multiply the magnification by the size difference.

So if this is what you can see:
(with x100 magnification)



and this is what you have drawn:



If the eyepiece lens is x10, and the objective lens is x10, then:

$$\text{Final magnification} = 10 \times 10 \times 2 \text{ (drawing is about twice the size of the specimen)} = \text{x200 magnification}$$

To work out the size of the original object under the microscope, measure a line across the drawing in mm, and divide it by the total magnification, giving the answer in μm .

Method B - Using the Eyepiece Graticule

- ▼ To do this, you need to have a scale (graticule) in position in your eyepiece, so that it can be seen when you look down the microscope. These scales are usually on small circular pieces of glass or acetate.
- ▼ To insert the scale in the eyepiece, remove the eyepiece lens from the microscope, and carefully unscrew the top lens. If you look down into the lens body, you should see a ledge running round the sides about halfway down. Drop the scale into the lens body, so that it rests on the ledge, then replace the top lens. It does not matter if the scale is the wrong way up, but if it annoys you, unscrew the lens again and turn the scale over.
- ▼ Look through the microscope, you should see the scale overlying the specimen.
- ▼ To calibrate the scale, you need to use a stage micrometer. This can be a special slide with a scale engraved on it, or one can be made by mounting a scale on a slide under a coverslip using Canada Balsam. It usually consists of a scale 1 cm long, which is divided into 100 units, each of which is 0.1 mm (100 µm), there is an extended line every 10th unit. It is worth being aware that these scales can be badly scratched.

To calibrate the eyepiece scale

- ▼ Place the stage micrometer on the microscope stage and hold it down with the clips.
- ▼ Using the eyepiece lens with the scale in, look through the microscope and focus it so you can see both scales clearly. This is usually easier if you focus the eye on the eyepiece scale and adjust the microscope so the stage scale comes into focus as well.
- ▼ Move the stage micrometer carefully so that the starting units of the two scales coincide.
- ▼ Count along the two scales until there is again a coincidence between the two scales. Note down the number of divisions along each of the two scales that this represents.
- ▼ As each division along the stage micrometer scale represents 100 µm, then:

$$1 \text{ division on the eyepiece scale (in mm)} = \frac{\text{Number of divisions on the stage micrometer scale}}{\text{Number of divisions on the eyepiece graticule scale}} \times 100$$

- ▼ At $\times 100$ and $\times 400$ magnification the lines on the scale will have a definite thickness. It is important to measure from one side of one scale mark, to the same side of the next coinciding mark.
- ▼ This procedure should be done for all the objective lenses on the microscope, so that you have a scale calibration for all magnifications. Record this information in a table with the following headings.

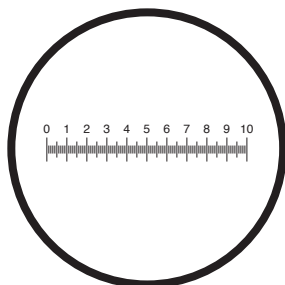
Power of lens	Magnification	Eyepiece scale 1 division (in mm)

- ▼ When you measure another specimen, you will already have the calibration figures, so all you have to do is count how many eyepiece scale divisions the specimen covers, and multiply that by the calibration factor for that objective lens.

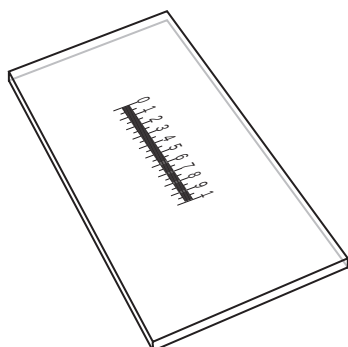
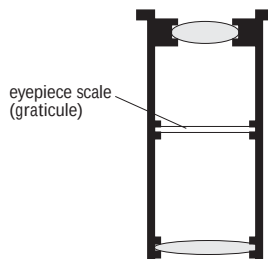
Now accurately measure as many structures as you can under the microscope using the calibrated eye piece graticule, and a range of different objective lenses.

Measurement of size under the microscope

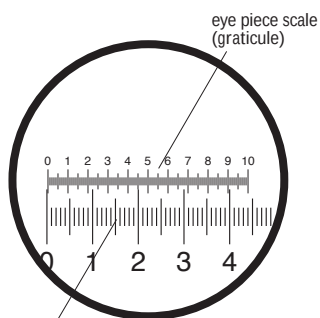
Eyepiece scale (graticule)



Eyepiece scale as seen when held up to the light away from the microscope.

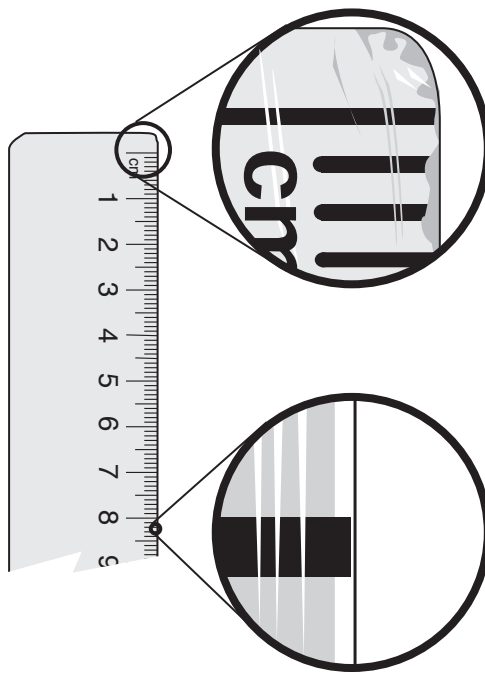


Scale in mm etched upon a microscope slide or a printed acetate sheet (slide micrometer)



Accurate measurements can be achieved using the two scales in combination through the lenses of the microscope.

More of the object is seen under low power objective lens



Less of the object is seen under high power objective lens

Teaching Notes

Individual help is often required to ensure that students fully understand the procedure involved in calibrating the eyepiece graticule. The diameter of red blood cells at about $7\mu\text{m}$ provides a good test of their measuring technique.

Requirements per student

- Microscope
- Eyepiece graticule
- Slide micrometer
- Suitable slides for measuring

Safety Considerations

There are no special safety considerations with this practical, other than the safe use of the microscope.

AS Module 1

(Biology and Human Biology)

Molecules, Cells and Systems

3 The properties of plasma membranes and the passage of substances through them

Water potential

Osmosis as the movement of water from a solution of less negative water potential to a solution of more negative water potential through a partially permeable membrane

An experiment to investigate the movement of water down water potential gradients through partially permeable membranes

Introduction

The changes in mass of tissue caused by water loss or gain, due to osmosis at the cellular level, are to be investigated. Pieces of plant food storage tissue, e.g. potato, will be placed in distilled water, and sucrose solutions of different concentrations.

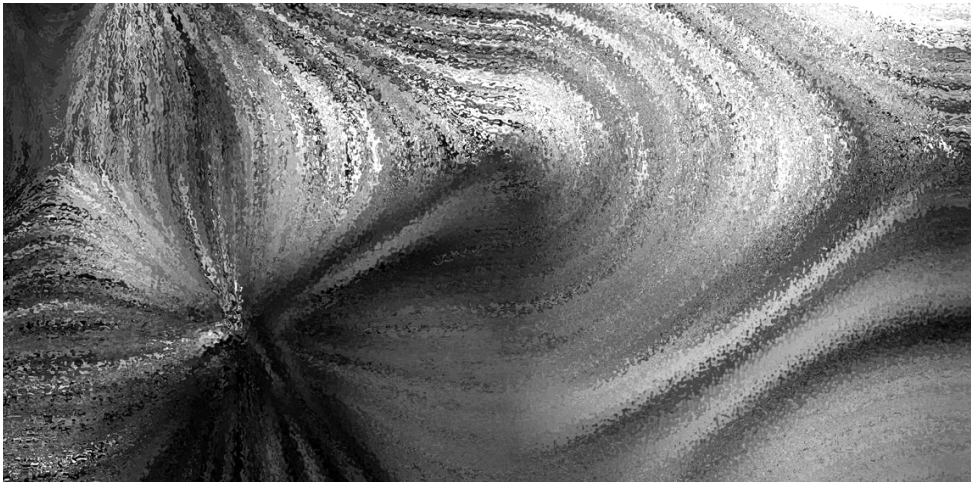
Method

- ▼ Collect a set of six 50cm³ beakers, and label them 0%, 0.5%, 1%, 5%, 10%, and 15%. Put 30 cm³ of distilled water in the 0% beaker.
- ▼ Using the 15% stock solution of sucrose supplied, make up the dilutions of sucrose solution listed, and put 30cm³ of each dilution in the appropriate labelled beaker.
- ▼ Collect a potato and use the cork borer to cut out a cylinder of potato tissue. Carefully cut the cylinder into discs 1mm thick, discarding the ends with the skin on. You will need about 60 discs.
- ▼ Using a balance, weigh 10 of the potato discs, note the mass and record in a suitable table. Repeat 5 times, so you have 10 discs of known combined mass for each solution.
- ▼ Add 10 discs of known combined mass to each of the solutions. Note the time.
- ▼ After 45-60 minutes, remove each set of discs from the beakers, very carefully blot them dry on a paper towel or tissue and reweigh them. Make a note of the new masses in the table.
- ▼ Calculate the % change in mass of the potato tissue in each liquid by dividing the gain or loss in mass by the original mass and multiplying by 100. Plot a graph of % change in mass against concentration of sucrose solution, include the distilled water result.

Discuss what the results indicate about osmosis and the effect that it has on living tissue.

Use the graph to estimate which concentration of sucrose solution possibly matches the internal concentration of the potato cells.

Questions



- 1 The cell as the basic unit of structure in prokaryotic and eukaryotic organisms**
- 2 The electron microscope and the technique of cell fractionation to study ultrastructure**
- 3 The properties of plasma membranes and the passage of substances through them**

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Question 1

- a) In the table below list three differences between the light (optical) and electron microscopes

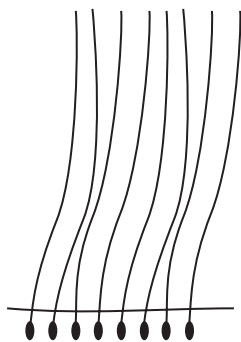
Light (optical) microscope	Electron microscope

(3)

- b) Describe the main difference between the principles of the transmission and scanning electron microscopes.

(2)

- c) Drawing X represents cilia. Drawing Y represents the appearance of a section of cilia.

**X****Y**

Interpret and explain the difference in appearance between the two cell diagrams.

(3)

Total 8

Question 1 MS

- a) In the table below list three differences between the light (optical) and electron microscopes

Light (optical) microscope	Electron microscope
Uses light	Uses a stream of electrons
Light focused by lenses	Electrons focused by magnets
Objects observed directly	Objects observed via fluorescent screen

(3)

- b) Explain the main difference between the principles of the transmission and scanning electron microscopes.

Transmission electron microscope - electrons pass through thin sections of specimen;

Scanning electron microscope - electrons reflected from surface of specimen;

(2)

- c) Drawing X represents cilia. Drawing Y represents the appearance of a section of cilia.

Interpret and explain the difference in appearance between the two cell diagrams.

cilia elongated relatively long thin structures;

thin sections along their length only cut small lengths;

and rarely 'catch' the connection to the cell;

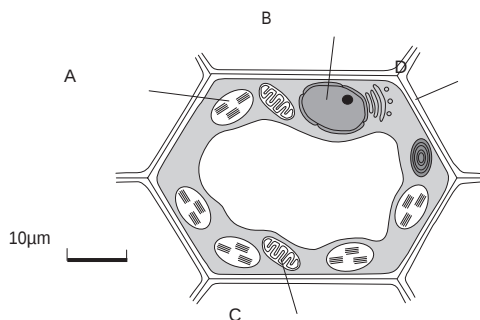
giving appearance of unattached segments;

(3)

Total 8

Question 2

Study the diagrams below of the generalised plant cell as seen under an electron microscope and answer the questions that follow.



- a) Identify structures A to C

A _____
B _____
C _____

(3)

- b) Typical plant cells are described as eukaryotic cells, describe the main features of eukaryotic cells.

(2)

- c) Name a type of organism with a prokaryotic cell, and name the cell structure they have in common with eukaryotic cells.

(2)

Total 7

Question 2 MS

Study the diagrams of the generalised plant and animal cells as seen under a light (optical) microscope and answer the following questions.

- a) Identify structures A to C

A = Chloroplast

B = Nucleus

C = Mitochondrion;

(3)

- b) Typical plant cells are described as eukaryotic cells, describe the main features of eukaryotic cells.

presence of a membrane bound nucleus at some stage in their existence;

membrane bound organelles, e.g. mitochondria;

(2)

- c) Name a type of organism with a prokaryotic cell, and name one cell structure that they have in common with eukaryotic cells.

bacterium;

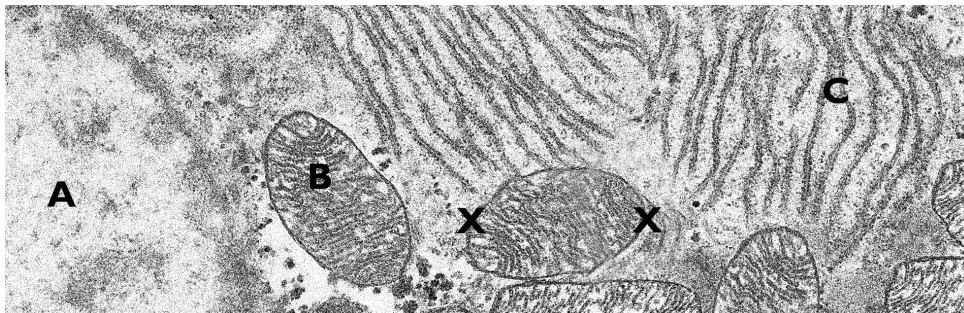
ribosomes;

(2)

Total 7

Question 3

The picture below shows part of an animal cell as seen under an electron microscope at a magnification of $\times 30\,000$, with all parts drawn accurately to scale.



- a) Identify structures **A** to **C**.

A _____

B _____

C _____ (3)

- b) Describe two ways in which a bacterial cell differs from the cell shown above.

i) _____

ii) _____

_____ (2)

- c) Calculate the actual size of structure C as measured between the two points marked X. Show your workings.

_____ (3)

- d) Name a structure in the drawing that is not visible even under the best light microscope.

_____ (1)

Total 9

Question 3 MS

The drawing shows part of a cell as seen under an electron microscope at a magnification of $\times 30\,000$, with all parts drawn accurately to scale.

- a) Identify structures A to C.
A nucleus;
B mitochondrion;
C rough or granular endoplasmic reticulum; (3)
- b) Describe two ways in which a bacterial cell differs from the cell shown above.
i) & ii) any two from
bacterium cell has no mitochondrion;
no nucleus;
no endoplasmic reticulum; (2)
- c) Calculate the actual size of structure C as measured between the two points marked X. Show your workings.
distance between points x-x = 30 mm;
magnification = $\times 30\,000$
therefore actual size = 30 divided by 30 000;
= 0.001 mm / 1.0 mm; (3)
- d) Name a structure in the drawing that is not visible even under the best light microscope?
ribosomes; (1)

Total 9

Question 4

Read the following passage and answer the questions that follow.

Neither the light microscope nor the electron microscope reveals much of the structure of the cell membrane. Models of membrane structure are constructed on the basis of the membrane's behaviour under certain conditions; in other words, it is impossible to separate structure and function since our knowledge of the structure derives from our knowledge of function.

Early investigations were based on studies of the rate of penetration of various materials into the cell, e.g. urea, sugars, alcohols, etc. It was found that fat-soluble substances penetrated more easily than others.

Lipids were extracted from red blood cell membranes, and the surface area that this lipid would have if it formed a layer one molecule thick was measured and found to be twice the surface area of the red blood cells.

- a) What does the fact that fat soluble substances enter the cell more rapidly than non-fat soluble substances, suggest about the nature of the membrane?

(1)

- b) What does the finding that the surface area of lipid extracted from the red cell membranes would have formed a layer one molecule thick with twice the surface area of the red blood cells, tell us about the arrangement of lipids in the red blood cell membrane?

(2)

- c) Further work indicated that 'smaller' molecules penetrated the membrane faster than would have been expected on the basis of their fat solubility alone.

What does the fact that smaller molecules penetrated the membrane faster than would have been expected on the basis of their fat solubility alone suggest about membrane structure?

(2)

- d) It has been claimed that all membranes have this common structure. Explain why the wide variety of functions shown by various membranes seems to argue against this.

(2)

- e) Why do neither the light microscope nor the electron microscope reveal much of the structure of the cell membrane.

(3)

Total 10

Question 4 MS

Neither the light microscope nor the electron microscope reveals much of the structure of the cell membrane. Models of membrane structure are constructed on the basis of the membrane's behaviour under certain conditions; in other words, it is impossible to separate structure and function since our knowledge of the structure derives from our knowledge of function.

- a) Early investigations were based on studies of the rate of penetration of various materials into the cell, e.g. urea, sugars, alcohols, etc. It was found that fat-soluble substances penetrated more easily than others.

What does the fact that fat soluble substances enter the cell more rapidly than non-fat soluble substances suggest about the nature of the membrane?

the membrane is fatty in nature;

(1)

- b) Lipids were extracted from red blood cell membranes, and the surface area that this lipid would have if it formed a layer one molecule thick was measured and found to be twice the surface area of the red blood cells.

What does the finding that the surface area of lipid extracted from the red cell membranes would have formed a layer one molecule thick with twice the surface area of the red blood cells tell us about the arrangement of lipids in the red blood cell membrane?

with the fats forming a bilayer;

suggests that there were two layers of lipid in the membrane;

arranged as a bimolecular lipid membrane;

(2)

- c) Further work indicated that 'smaller' molecules penetrated the membrane faster than would have been expected on the basis of their fat solubility alone.

What does the fact that smaller molecules penetrated the membrane faster than would have been expected on the basis of their fat solubility alone suggest about membrane structure?

that the membrane is porous;

allowing smaller molecules to penetrate faster through pores of a certain diameter;

independent of their fat solubility therefore pores are 'aqueous';

(2)

- e) It is claimed that all membranes showed this common structure. Explain why the wide variety of functions shown by various membranes seems to argue against this.

function to a large part determined by structure and vice versa;

some functions so different as to require different membrane structure;

(2)

- f) Why do neither the light microscope nor the electron microscope reveal much of the structure of the cell membrane.

the resolving power of the LM is insufficient;

the resolving power of the EM is insufficient;

preparation techniques for the EM are too drastic to leave the membrane unaltered;

molecular level too small to be revealed;

(3)

Total 10

Question 95

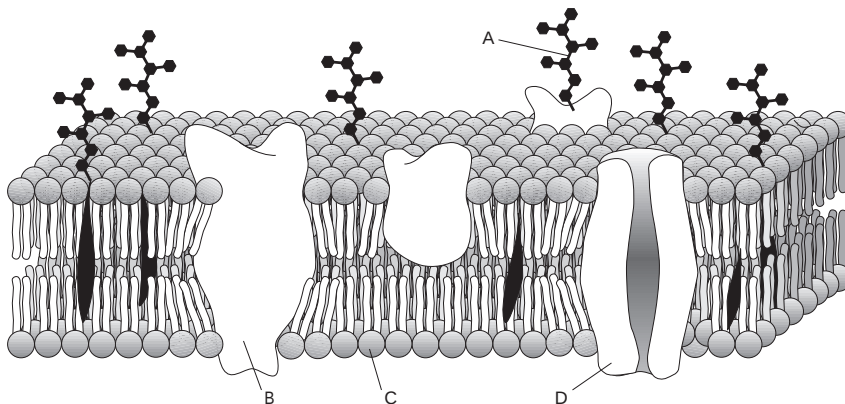
Give an account of the structure, properties and functions of the cell surface membrane.

- 1 'fatty' in nature, two layers of phospho-lipids bilayer);
- 2 hydrophobic poles together, hydrophilic poles 'outwards';
- 3 stabilised by cholesterol;
- 4 surface proteins / glycoproteins for recognition and communication;
- 6 glycolipids;
- 6 embedded proteins, some enzymes catalysing membrane reactions;
- 7 some carriers involved with active transport across the membrane;
- 8 some 'pore' proteins allowing passage of water soluble solutes;
- 9 the fluid mosaic model;
- 10 partially permeable selective barrier to the passage of materials into and out of cells;
- 12 maintains the integrity of the contents;
- 13 can be involved in movement e.g. pseudopodia in white cells;
- 14 endocytosis and exocytosis;

Total 10

Question 6

- a) Label the structures A, B, C, and D on the simplified diagram of the cell surface membrane shown below



(4)

- b) Describe why phospholipids always form bilayers in aqueous systems.

(2)

- c) Describe the roles of the following components of the cell surface membrane.

- i) cholesterol

(1)

- ii) glycoproteins

(1)

- iii) carrier proteins

(1)

- d) Give a brief description of the transport of substances through the cell surface membrane by endocytosis and exocytosis.

(4)

Total 13

Question 6 MS

- a) Label the structures A, B, C, and D on the simplified diagram of the cell surface membrane shown below.
- A** Glycoprotein;
B Transmembrane protein;
C Phospholipid;
D Pore protein; (4)
- b) Describe why phospholipids always form bilayers in aqueous systems.
phospholipids have hydrophilic and hydrophobic poles;
hydrophobic poles 'attract' each other; (2)
- c) Describe the roles of the following components of the cell surface membrane.
- i) cholesterol
stabilises the lipid bilayer; (1)
- ii) glycoproteins
cell surface recognition / communication; (1)
- iii) carrier proteins
transport of substances into and out of cell; (1)
- c) Give a brief description of the transport of substances through the cell surface membrane by endocytosis and exocytosis.
endocytosis involves 'ingesting' a volume of the exterior solution;
enclosing it within a vacuole within the cell;
exocytosis involves a vacuole / vesicle reaching cell surface membrane from cell interior;
membrane of vesicle coalesces with cell surface membrane;
contents secreted to exterior; (4)

Total 13

Question 7

Fick's law states that diffusion rate is proportional to:

surface area x difference in concentration/ thickness of exchange surface

a) Describe how each of the factors in the equation affects the rate of diffusion:

i) surface area

(1)

ii) difference in concentration

(1)

iii) thickness of exchange surface

(1)

b) What feature of a mitochondrion increases its surface area for diffusion?

(1)

c) Explain how an increase in the aerobic activity of a cell increases the diffusion gradients of oxygen and carbon dioxide between the cytoplasm of the cell and the internal matrix of a mitochondrion.

(4)

Total 8

Question 7 MS

Fick's law states that diffusion rate is proportional to:

surface area x difference in concentration/ thickness of exchange surface

a) Describe how each of the factors in the equation affects the rate of diffusion:

i) surface area

the larger the surface area the faster the diffusion rate;

(1)

ii) difference in concentration

the larger the difference in concentration the faster the diffusion rate;

(1)

iii) thickness of exchange surface

the thicker the exchange surface the slower the diffusion rate/ vice versa;

(1)

b) What feature of a mitochondrion increases its surface area for diffusion?

the folding(s) of the internal membrane / cristae;

(1)

c) Explain how an increase in the aerobic activity of a cell increases the diffusion gradients of oxygen and carbon dioxide between the cytoplasm of the cell and the internal matrix of the mitochondrion.

as the activity of a cell increases more ATP required;

more oxygen used;

and more carbon dioxide produced;

in aerobic respiration in the internal matrix of the mitochondrion;

difference between the levels of these two gases in the matrix of the mitochondrion and the cytoplasm of the cell increased;

(4)

Total 8

Question 8

a) Describe what is meant by the following terms with regard to the uptake of substances by cells:

i) facilitated diffusion

(2)

ii) active transport

(3)

b) Explain why, when respiration is inhibited in cells bathed in pure water, substances, especially ions, are released from the cells.

(4)

Total 9

Question 8 MS

a) Describe what is meant by the following terms with regard to the uptake of substances by cells:

i) facilitated diffusion

diffusion down a diffusion gradient;

from higher concentration to lower concentration;

speeded up/ made easier by the presence of special carriers in the membrane;

(2)

ii) active transport

transport against the prevailing diffusion gradient;

from lower concentration to higher concentration;

involving carriers in cell membrane;

using energy from respiration / ATP;

(3)

b) Explain why, when respiration is inhibited in cells bathed in pure water, substances, especially ions, are released from the cells.

in pure water the diffusion gradient for ions is from the more concentrated cell contents to the pure water;

this situation is maintained by active ion uptake;

using energy from respiration / ATP;

if respiration is inhibited active uptake is reduced and substances diffuse out of the cell into the surrounding water;

if respiration inhibited completely, membrane breaks down and all cell contents are released into the surrounding water;

(4)

Total 9

Question 9

Read the following passage and answer the following questions.

Osmosis can be described as the movement of water from a solution of less negative (higher) water potential to a solution of more negative (lower) water potential through a partially permeable membrane. It can also be thought of as the special case of the diffusion of water, with water molecules diffusing down a gradient from regions of its higher potential to regions of its lower potential through a partially permeable membrane. The term 'potential' is used rather than the more familiar 'concentration' when talking about diffusion, as water is the solvent. Like diffusion, the rate of the passage of water by osmosis is proportional to the steepness of the potential gradient. Pressure increases the water potential of a given aqueous system such as a living plant cell.

- a) i)** What is the water potential of pure water at standard temperature and pressure?

(1)

- ii)** Explain why alternatives to the term the 'concentration of water' have been devised.

(2)

- iii)** Name two factors (other than the one mentioned in the text) to which the rate of osmosis is proportional to.

(2)

- b)** If a plant cell is placed in pure water, it eventually becomes fully turgid, the cellulose cell wall can expand no further, and so no more water enters the cell. Explain this process in terms of the effect of pressure on water potential.

(4)

Total 9

Question 9 MS

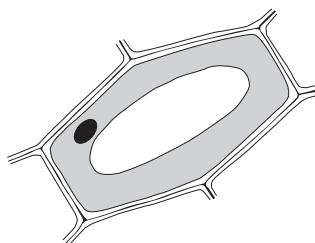
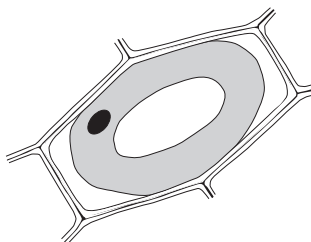
Read the following passage and answer the following questions.

- a) i) What is the water potential of pure water at standard temperature and pressure?
0 (nought / zero) (1)
- ii) Explain why alternatives to the term the 'concentration of water' have been devised.
water is the solvent in which solutes dissolve;
the term concentration normally refers to the solute;
therefore the 'term concentration of water' implies it is acting as a solute; (2)
- iii) Name two factors (other than the one mentioned in the text) to which the rate of osmosis is proportional.
temperature;
surface area over which it is occurring; (2)
- b) If a plant cell is placed in pure water, it eventually becomes fully turgid, the contents of a fully turgid cell push out against the stretched cell wall which can expand no further and so no more water enters the cell. Explain this process in terms of the effect of pressure on water potential.
the pressure inside the cell is raised above that in a non-turgid cell;
an increase in pressure increases the water potential;
reducing the water potential gradient to zero;
so no more water enters the cell (4)

Total 9

Question 10

The diagrams below show two plant cells, each bathed in a different external solution.

**Cell A****Cell B**

- a) i) Name the cell which is in a solution more dilute than the cell contents.

(1)

- ii) Describe the appearance of the cell which is in a solution more concentrated than the cell contents.

(2)

- b) Describe and explain what would happen to animal cells e.g. human red blood cells if they are placed in a solution with a water potential greater than their contents?

(3)

Total 6

Question 10 MS

The diagrams below show two plant cells, each bathed in a different external solution.

- a) i) Name the cell which is in a solution more dilute than the cell contents.
cell A; (1)
- ii) Describe the appearance of the cell which is in a solution more concentrated than the cell contents.
the cell membrane is withdrawn from the cell wall;
the central vacuole is smaller than in cell A; (2)
- b) Describe and explain what would happen to animal cells e.g. human red blood cells if they are placed in a solution with a water potential greater than their contents?
water would enter by osmosis (endosmosis);
cells would increase in volume (swell);
cell membrane would be stretched;
and burst if water potential gradient high enough; (3)

Total 6

Question 11

The data below are from an experimental investigation on osmosis carried out by a student using cylinders of well washed beetroot tissue from which no pigment was leaking.

Immersed in	Initial mass (g)	Mass (g) after 30 minutes	% difference
distilled water	2.81	2.97	+5.70
0.3 M sucrose solution	2.79	2.89	+3.60
1.0 M sucrose solution	2.78	2.60	-6.10

Where M = molarity which is a measure of concentration.

- a) Explain the figures in the table in terms of osmosis for the cells immersed in each of the following.

- i) Distilled water.

(3)

- ii) 0.3 M sucrose solution.

(1)

- iii) 1.0 M sucrose solution.

(2)

- b) Explain the following results, observed after the cylinders of beetroot tissue from the above experiment were subjected to the following treatments.

- i) When placed in distilled water they all showed a gain in mass of around 5.7% compared to their original mass.

(1)

- ii) When gradually brought to the boil, red pigment flooded into the distilled water.

(2)

- c) One of the problems with experiments in which changes of mass of tissues are measured is that relatively large amounts of water are held in the cellulose cell wall. Explain why this gives an inaccurate picture of water movement by osmosis in experiments such as the one described.

(3)

Total 12

Question 11 MS

The data below are from an experimental investigation on osmosis carried out by a student using cylinders of well washed beetroot tissue from which no pigment was leaking.

- a) Explain the figures in terms of osmosis in:
- i) distilled water,
cell contents have a lower water potential than distilled water;
as a result of dissolved solutes;
water enters through partially permeable cell surface membrane by osmosis; (3)
 - ii) 0.3 M sucrose solution,
the water potential gradient operates in the same direction but is less steep; (1)
 - iii) 1.0 M sucrose solution,
1.0 M sucrose solution has a lower water potential than the cell contents;
therefore water leaves the cell by osmosis; (2)
- b) Explain the results after the cylinders of beetroot tissue from the above experiment were then subjected to the following treatments.
- i) When placed in distilled water they all showed a gain in mass of around 5.7% compared to their original mass.
effects of osmosis reversible;
therefore showed gains similar to cylinder in distilled water originally; (1)
 - ii) When they are gradually brought to the boil, red pigment flooded into the distilled water.
membrane structure broken down by heat;
therefore becomes fully permeable to pigment which floods out; (2)
- c) One of the problems with experiments in which changes of mass of tissues are measured is that relatively large amounts of water are held in the cellulose cell wall. Explain why this gives an inaccurate picture of water movement by osmosis in experiments such as the one described.
cellulose cell wall completely permeable;
outside the cell surface membrane;
plays no part in osmosis but contributes to changes in mass; (3)

Total 12